PATHOPHYSIOLOGY OF FASCIOLA HEPATICA INFECTIONS IN RABBITS

A Thesis Submitted for the Degree of Doctor of Philosophy

in the Faculty of Veterinary Medicine

of the University of Glasgow by

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CONTENTS

VOLUME II

GENERAL INTRODUCTION

Fasciola hepatica is a common parasite of sheep and cattle and has also been found in pigs, horses, rabbits and man. The adult parasite, which is found in the bile ducts of the host's liver is hermaphrodite and produces large numbers of eggs which enter the intestine via the bile and pass out in the faeces. These eggs subsequently hatch and motile ciliated miracidia emerge. If within the following 24 hours contact is made with a suitable host snail (in Britain, mainly Lymnaea truncatula), the miracidium bores into the soft tissues of the snail where it develops into cercariae. These emerge from the snail, cast off their tails and encyst on the herbage.

Infection of a suitable host follows ingestion of these cercariae. After excysting in the intestine the larval forms penetrate the wall of the gut and migrate across the abdominal cavity to the liver which they enter by boring through the capsule. Thereafter the young flukes wander in the liver parenchyma and finally enter the bile ducts in which they become mature about 8 or 9 weeks after infection.

Although death of the host may occur during this acute or migratory stage of the disease due to extensive destruction of liver tissue or haemorrhage from this organ, the chronic form which is usually characterised by anaemia and hypoproteinaemia is more commonly encountered.

Views on the aetiology of the changes in blood composition which accompany chronic infections with F. hepatica are necessarily associated

with one's ideas on the feeding habits and nutrition of the parasite. Broadly speaking there are two schools of thought on the problem. Some authors consider that the parasite lives mainly on blood and that the anaemia and hypoproteinaemia shown by the host are primarily due to direct blood loss. Others do not consider that the liver fluke is haematophagic and therefore feel that blood loss cannot be the main factor in bringing about the pathological changes in the host.

At the present time most of the work that has been carried out on the clinical pathology of fascioliasis has been directed towards establishing the type and extent of e.g. the plasma protein changes that occur as a result of infection with this parasite, and no results have been reported on the changes in the total pools of circulating proteins. Furthermore when one considers that the level of any blood constituent, whether it be albumin or red cells is basically determined by the relative rates of synthesis and catabolism of that constituent, it is clear that although much useful information may be obtained about the pathogenic effects of parasites on their host by the application of conventional analytical techniques such as measurement of the levels of albumin and haemoglobin in the blood of infected animals, it is completely impossible to study the kinetics of the systems involved without resorting to isotope techniques.

With the advent of radioisotopically labelled plasma proteins and red cells the metabolism of blood constituents may now be studied in vivo. In this way, data of considerable clinical importance such as the amount of a given substance e.g. albumin which is available for metabolic

purposes and its rate and site of degradation within the body can be obtained.

Jennings, Mulligan and Urquhart (1956) first applied radioisotopic techniques to study the anaemia of F. hepatica infections. These authors injected ^{32}P-labelled red cells and ^{131}I-labelled albumin into fluke-infected and normal rabbits and from the radioactivity in blood and flukes 1 hour later, made an estimate of the quantity of blood consumed by the parasites. The figure obtained was 0.2 ml. of blood/day/fluke. The difficulty with this type of experiment is that normal bile, in addition to containing phosphate is one of the pathways for iodine excretion and it is therefore difficult to assess to what extent the radioactivity of the flukes was due to uptake from the bile of labelled breakdown products of either the cells or the plasma protein. However since the radioactivity of the flukes was in all cases about 30 times that of the bile it was considered that the activity of the flukes would be largely attributable to blood consumption. In any case the results of these experiments provided evidence that the anaemia (and hypoproteinaemia) associated with chronic fascioliasis was due to the passage of blood into the gastrointestinal tract via the bile and gave further support to the theory that the adult parasite is haematophagic.

The alternative to direct analysis of liver flukes is to study the metabolism of red cells and plasma proteins in the host animals and to determine whether the changes observed in infected animals are consistent with a blood loss brought about by the parasite. This is the approach which is embodied in the experiments described in this thesis.

It should however be pointed out that although these investigations mainly involved the use of isotopically labelled plasma proteins and red cells, conventional biochemical analyses of the type previously mentioned were simultaneously carried out in order that a deeper appreciation of the effects of F. hepatica infections on the metabolism of these blood constituents could be obtained.

In the first section, radioiodinated albumin and IgG immunoglobulin were used to assess the relative importance of alterations in either the rate of synthesis and/or catabolism of these proteins as being the primary influence in causing the often marked hypoalbuminaemia and hypergammaglobulinaemia usually associated with chronic infections. The importance of these factors in the aetiology of the anaemia found in rabbits harbouring populations of adult parasites was determined using ^{51}Cr and ^{59}Fe-labelled red cells (Section 2).

Because of the likelihood that any increase in the rate of degradation of plasma proteins and red cells was caused by haemorrhage into the gut as a result of the blood-sucking activities of the parasite, the daily faecal radioactivity of each animal was determined throughout these and subsequent studies and compared with that of the appropriate blood or plasma sample to obtain some measure of such losses.

If anaemia and increased degradation of plasma proteins are in fact caused by the passage of blood into the gastrointestinal tract of infected animals, then removal of the flukes by treating infected rabbits with anthelmintic should result in cessation of this haemorrhage and a

rapid return to normal P.C.V. values within a short period of time. This hypothesis was tested by measuring the movement of plasma macromolecules and red cells into the gut of rabbits chronically infected with F. hepatica before and again following anthelmintic treatment, and relating any differences observed to changes in venous haematocrit. The results of these studies are reported in Section 3.

Although the simplest explanation of the observed changes in blood composition of fluke-infected animals is that the parasite sucks whole blood, damage to the walls of the bile ducts may be such that a preferential loss of plasma occurs between epithelial cells. In order to define the relationship between plasma and red cell loss, and the effect of anthelmintic treatment upon them, an experiment was carried out (the results of which are reported in Section 3), in which plasma and red cell losses were simultaneously measured using ^{95}Nb-labelled albumin and ^{51}Cr-labelled red cells.

It must be made clear that all the experiments described in these Sections were carried out on animals harbouring a population of fully mature parasites. To find out whether the straightforward blood loss explanation is not further complicated by impairment of synthetic processes i.e. protein synthesis and/or erythropoiesis during the early stages of the disease, e.g. when the flukes are migrating through the liver, and also animals with long-standing infections, the metabolism of labelled albumin and red cells were simultaneously studied throughout the disease process. In this way, it was hoped that a comparison of the pathogenic effects of F. hepatica infections on the host at different stages

of infection could be made.

GENERAL MATERIALS AND METHODS

(i) Experimental Animals

Adult rabbits of the Dutch or New Zealand White variety were used in these studies and received pelleted diet No. SG.1 (Oxoid Ltd., London).

(ii) Collection of Urine and Faeces

During the experiments the animals were housed in metabolism cages fitted with double collecting grids (Fig. 1). Faeces were collected on a gauze sheet supported by the lower grid. In this way contamination by urine was minimised since the total daily output of faeces tended to be spread over the whole area of the grid. The total faecal output for each 24-hour collection period was weighed, spread out on paper, and two random 5 gm. samples were packed to a volume of 5 ml. in counting tubes for radioactivity determinations. Daily urine output was measured, and duplicate 5 ml. samples pipetted into counting tubes and assayed for radioactivity.

(iii) Infection of Rabbits

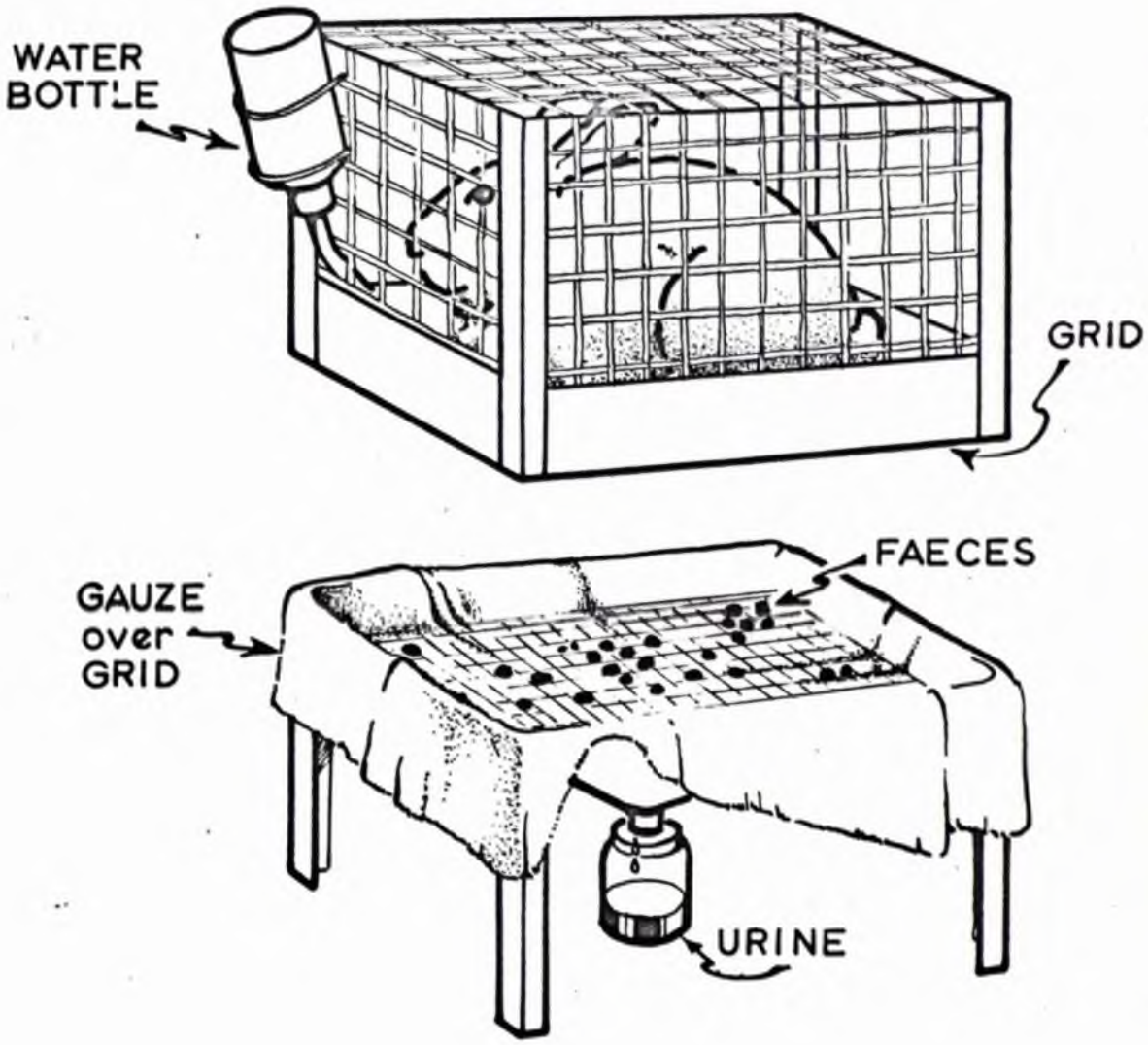
Metacercariae were kindly supplied by Dr. J. Armour of the Wellcome Laboratories for Experimental Parasitology, Glasgow. The appropriate numbers encysted on "cellophane" were transferred to gelatin capsules and administered by mouth. Some rabbits were infected with 100 metacercariae, others with 50, depending upon the weight of the animal.

(iv) Treatment with Anthelmintic

The anthelmintic used in the treatment experiments was oxyclozanide

MODIFIED METABOLISM CAGE

FIGURE 1



("Zanil", I.C.I. Pharmaceutical Division). A single dose of 17 mg/kgm. was administered by stomach tube.

(v) Autopsy Procedure

Rabbits were killed by intravenous injection of sodium pentobarbitone ("Nembutal", Abbot Laboratories, Queenborough, Kent), the abdomen opened and liver removed. The large extrahepatic bile ducts, which usually contained most of the parasites, were opened and the flukes removed. The lobes of the liver were also dissected and squeezed to remove the few flukes present in the intrahepatic ducts.

(vi) Haematological Methods

(a) Packed Cell Volume (P.C.V.)

The packed cell volume percentage was determined by the micro-haematocrit method. Capillary tubes containing the blood sample were sealed by heat at one end and centrifuged for 5 minutes in a micro-haematocrit centrifuge (Hawksley and Son Ltd.). The percentage P.C.V. was determined from the scale on a Hawksley Micro-Haematocrit Reader

(b) Haemoglobin Concentration (Hb)

Blood haemoglobin was determined by the cyanmethaemoglobin method of van Kampen and Zijlstra (1961). 0.02 ml. of well mixed blood was added to 5 ml. dilute potassium ferricyanide solution. Haemoglobin was thus oxidised to haemiglobin which in turn was converted by treatment with cyanide to the stable cyanmethaemoglobin. This compound was measured colorimetrically at 542 mμ, and the concentration of haemoglobin (gm./100 ml. blood) determined with the aid of a standard cyanmethaemoglobin solution.

(c) Red Cell Count

Red Cell counts (x 10^6/cu.mm.) were determined by an electronic red cell counter, (Model B, Coulter Electronics Ltd., Dunstable, Beds.).

(d) Serum Iron

For these determinations, blood samples were allowed to clot in iron-free universal bottles and the serum obtained similarly stored. Serum iron was determined by the method of Ramsey (1957). Serum was heated with 2,2' - dipyridyl at pH 4.5 and sodium sulphite as reducing agent. Addition of chloroform coagulated the proteins, and after their removal by filtration, the pink colour of the ferrous dipyridyl complex ion was measured at 520 mμ.

(vii) Serum Protein Analysis

(a) Total Protein Concentration

Total serum protein concentration was estimated by the biuret method of Weichselbaum (1946).

(b) Serum Protein Fractionation

Separation of the serum protein fractions was carried out by electrophoresis. Cellulose acetate strips (Oxoid Ltd., London) were saturated with barbitone buffer (pH 8.6), lightly blotted to remove excess buffer and laid across the supports of an electrophoresis tank (Shandon Scientific Co. London). 0.003 ml. serum was applied to the strip about 4 cm. from the cathode end, using a micro-pipette. A constant voltage of 150 v was applied for 1 hour from a Vokam power pack (Shandon Scientific Co. Ltd.).

The strips were removed, dried in a hot air oven to "fix" the proteins and developed by staining with 0.2% Ponceau S (G.T. Gurr Ltd., London) in 3% aqueous trichloracetic acid for 5 minutes. The strips were evaluated automatically as described by Neill (1963) using a Chromoscan recording densitometer (Joyce Loebl and Co. Ltd., Gateshead, England). The results were expressed as gm. of albumin, total globulin, alpha, beta and gammaglobulin per 100 ml. of serum.

(viii) Statistical Methods

Statistical methods employed were those described by Snedecor (1956) and Bishop (1966). Half-life values quoted were calculated by regression analysis. Unless otherwise stated in the text, correlation coefficients of activity against time were very highly significant ($r > -0.95$). Standard deviations and P values (Student's t - test) are quoted. A value equal to or less than 0.05 is regarded as being statistically significant.

(ix) Preparation of Albumin for Labelling

Albumin was separated from pooled normal rabbit serum by one of the following procedures:

(a) Salt Fractionation

Most of the globulins were precipitated by the addition of 18 gm. of anhydrous sodium sulphate per 100 ml. of serum. Two precipitations were carried out and the final supernatant dialysed against 0.85% NaCl until no trace of sulphate could be detected in the dialysate with barium chloride. This preparation contained a little α-globulin, but was thought to be suitable for the comparative experiments envisaged.

(b) Molecular Sieve Chromatography

Pre-swollen Sephadex G 100 (Pharmacia Fine Chemicals, Uppsala, Sweden) is a cross-linked dextran gel, which when packed in a chromatographic column separates the serum proteins into two main fractions on the basis of molecular weight. Large molecular weight proteins, i.e. globulins, pass unhindered through the column, while albumin (M.Wt. 69,000) enters the gel particles and is retarded.

Albumin was thus separated from the globulins by passing 20 ml. samples of normal rabbit serum through a Sephadex G 100 bed (2.5 x 130 cm.). Five ml. portions of the eluate were collected and the protein distribution determined by measuring the optical density at 280 mμ (Fig. 2). Fractions corresponding to the albumin peak were bulked, dialysed overnight against saline and freeze-dried. In all cases, albumin preparations were dissolved in 0.85% NaCl prior to labelling.

(x) Preparation of 7S-gammaglobulin for Labelling

This fraction was a purified product obtained from the Nann Research Laboratories Inc., New York. It is specified as being 100% pure 7S rabbit gammaglobulin by immunoelectrophoresis. This preparation was dissolved in 0.01 M phosphate buffer for labelling with radioiodine.

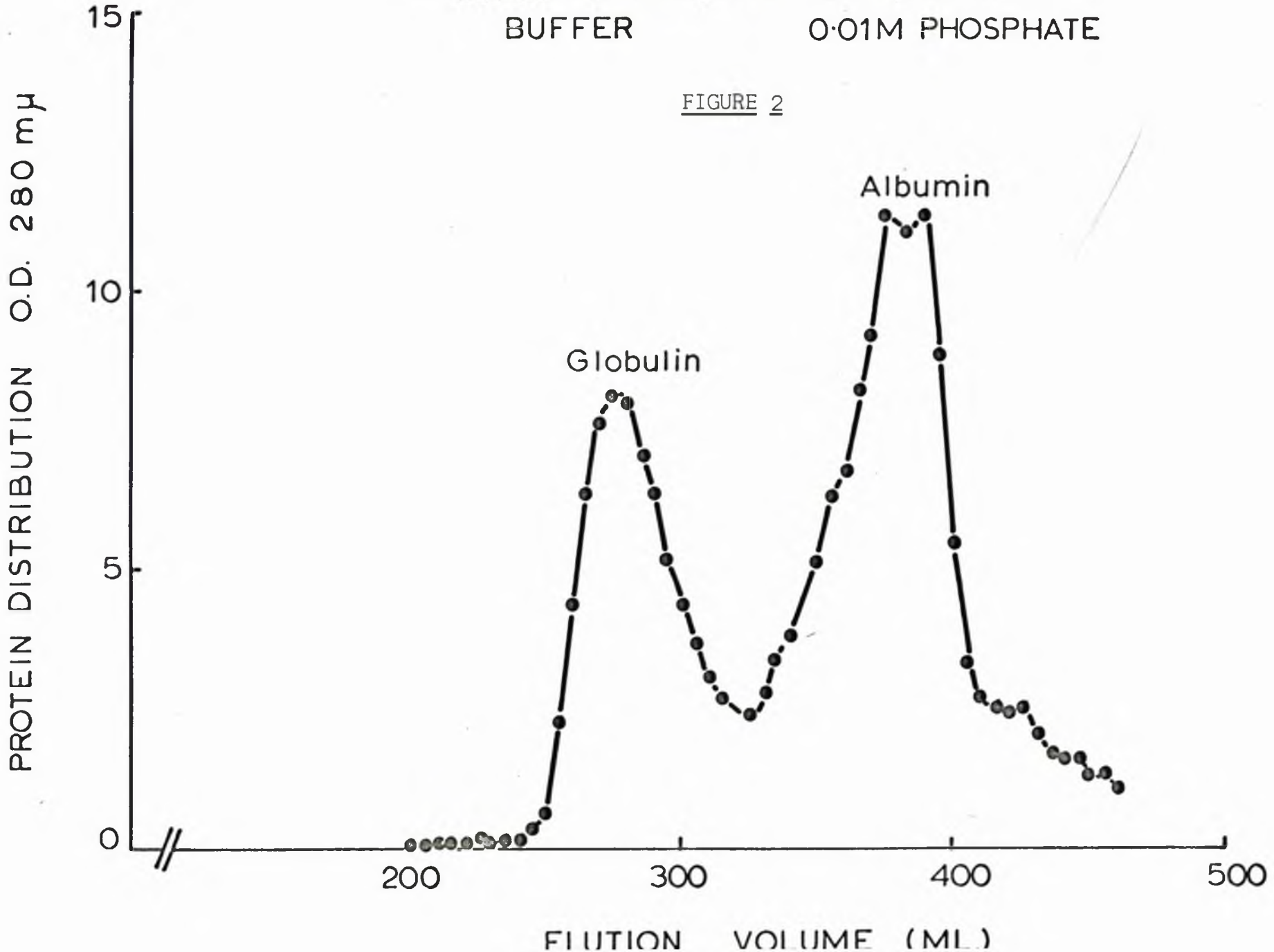
(xi) Trace-iodination of Proteins with ^{131}I or ^{125}I

The reaction of iodine with proteins results in the oxidation of sulphydryl groups, substitution in tyrosine residues, and, if large amounts of iodine are present, direct addition to other residues, e.g. histidine, this latter reaction leading to denaturation of the protein. The

PROTEIN DISTRIBUTION FOLLOWING G-100 SEPHADEX GEL FILTRATION of NORMAL RABBIT SERUM (20ML.)

COLUMN DIMENSIONS 2·5×130cm
BUFFER 0·01M PHOSPHATE

FIGURE 2



incorporation of iodine into the protein molecule must therefore be controlled in order that only tyrosine residues are iodinated. Labelling is usually carried out by treating the protein in slightly alkaline solution with "carrier" iodine or iodine monochloride to which has been added a solution of radioactive iodine (as carrier-free iodide). The preparation of the radioactive form depends on an exchange reaction between the isotope and one of the above "carriers".

A further requirement is that the radioiodine must first be converted into an electrophilic form, i.e. into positive iodine, as present in hypoiodous acid (IOH). This can be achieved by treatment of iodine or iodine monochloride with either water or base.

viz.

$$I_2 + H_2O \longrightarrow H_2OI + I^- \qquad \text{Reaction 1.}$$

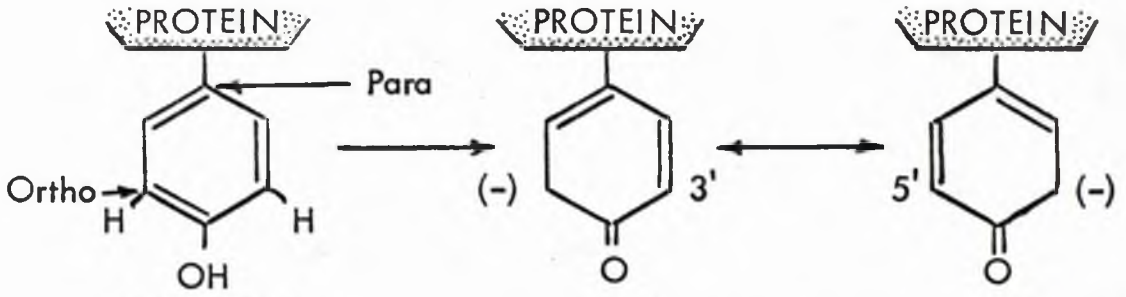
$$I_2 + OH^- \longrightarrow IOH + I^- \qquad \text{Reaction 2.}$$

$$ICl + OH^- \longrightarrow IOH + Cl^- \qquad \text{Reaction 3.}$$

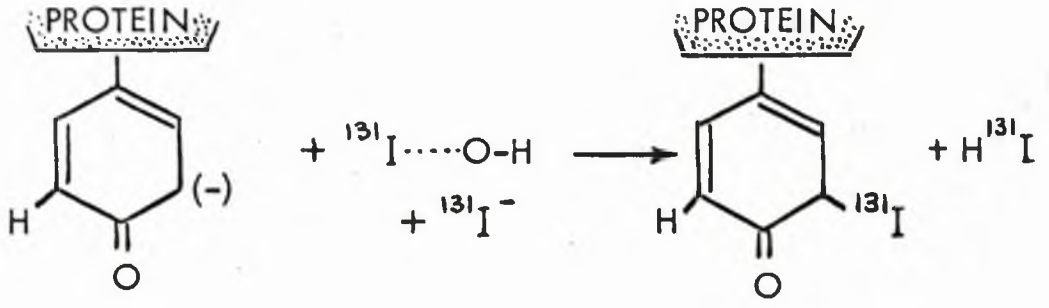
Reactions must be carried out in solution, and since iodine is only very sparingly soluble in water, reaction 1 is not a practical proposition. However, a convenient reagent for iodinating proteins is potassium iodide (KI), since in solution, it forms stable complexes with iodine. Earlier methods of iodination involved the use of KI as "carrier", and really differed only in the base used to liberate the hypoiodous acid. Hughes and Streeslo (1950) iodinated protein solutions in bicarbonate buffer, while Francis, Mulligan and Wormall (1951) used ammoniacal protein solutions.

FIGURE 3

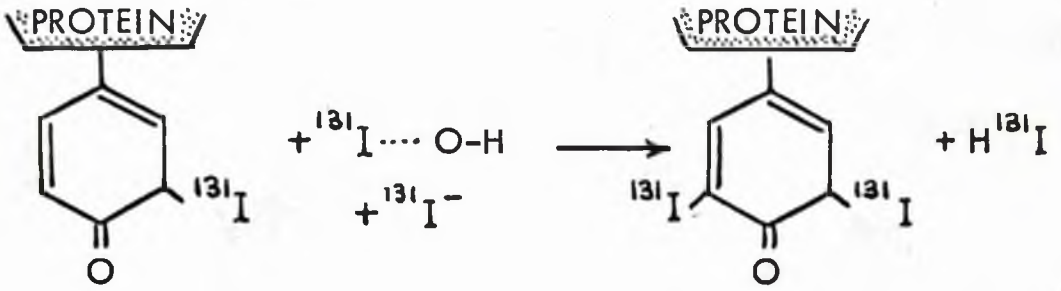
MECHANISM OF IODINE SUBSTITUTION ON TYROSINE



(A) Formation of 3' or 5' MONO-IODOTYROSINE



(B) Followed immediately by substitution of I for H on remaining ortho position forming 3', 5' DI-IODOTYROSINE



A solution of iodine in potassium iodide forms an iodine/iodide equilibrium in which added radioiodine can also take part i.e.

$$I_2 + KI + {}^{131}I \longrightarrow K{}^{131}I_3 \rightleftharpoons {}^{131}I_2 + {}^{131}I \qquad \text{Reaction 4.}$$

Thus, from the point of view of obtaining maximum utilisation of the radioiodine, the amount of "carrier" KI present should be kept to a minimum. On addition of this mixture to an alkaline solution of the protein, the radioiodine is converted to hypoiodous acid and free iodide i.e.

$$2\,{}^{131}I_2 + OH^- \longrightarrow {}^{131}ICH + {}^{131}I^- \qquad \text{Reaction 5.}$$

Since only ^{131}ICH can react with the protein (the radioiodide takes no part in the iodination), this reaction means that the theoretical maximum utilisation of the radioiodine is 50%.

Figure 3 outlines the reactions of hypoiodous acid with tyrosine. Since the protein is dissolved in a buffer at alkaline pH, the tyrosine is present as phenoxide anions. Radioiodine, in contact with buffer, is converted to positive iodine which attacks the negatively charged positions of the benzene nucleus.

The net result is formation of 3:5 di-iodotyrosine and acidic $H^{131}I$. It is therefore apparent why the protein must be adequately buffered. Since two molecules of hypoiodous acid are required to complete the substitution, the theoretical maximum yield using KI as "carrier" is 25%. In practice however, yields of about 16% are usually obtained due to the oxidation of sulphydryl groups which occurs before substitution reactions occur. To improve the yield, it would be necessary to convert the iodide to iodine which could then attach to the protein by substitution, e.g. by the use

of an oxidising agent i.e.

$$2H^{131}I + Cl_2 \longrightarrow 2HCl + {}^{131}I_2 \qquad \text{Reaction 6.}$$

Other oxidising agents used are ammonium persulphate (Gilmore, Robins and Reid, 1954), and hydrogen peroxide (McFarlane, 1958). The use of these agents results in some degree of protein denaturation, and should therefore be avoided. Alternatively, prior oxidation of sulphydryl groups could be carried out e.g. by the addition of iodine to the protein solution at pH 4 - 5, (when the tyrosine is not ionised). The removal of the excess iodine by ion-exchange chromatography carries some risk of surface denaturation to the protein.

The use of iodine monochloride to trace-label proteins was first described by McFarlane (1958). It has become widely accepted as the most suitable form of "carrier" because of its stability in aqueous solutions, low volatility, and because the iodide is theoretically 100% available for substitution in the benzene ring of tyrosine. The need for radioiodine completely free from reducing agents requires that the preparation is uncontaminated by thiosulphate. For this purpose ^{131}I or ^{125}I condensed into $^{N}/_{50}$ NaOH can be obtained from the Radiochemical Centre, Amersham.

Thiosulphate-free radioiodine is added to a dilute aqueous solution of iodine monochloride which is then converted to hypoiodite (Reaction 3). This conversion is carried out by adding a glycine buffer of pH 8.5 to the iodine monochloride solution. This step, which is indicated by loss of the characteristic yellow colour of ICl appears to be a prerequisite for substitution of iodine in the benzene ring of tyrosine.

This is then immediately rapidly mixed with the protein solution similarly buffered at pH 9. Provided the molar ratio of iodine monochloride to protein is greater than 2, 60 - 80% of the radioiodine becomes bound to the protein. Lower efficiencies are obtained using lower molar ratios because of preferential utilisation of the iodine monochloride for non-specific oxidations, and especially of sulphydryl. If sulphydryl groups are oxidised prior to the labelling, 90% utilisation of the isotope can be achieved by this method. The ICl method of McFarlane was used for all radioiodine labelling of proteins in the work described in this thesis.

(a) Preparation of Iodine Monochloride

The method of preparation followed that given by Vogel (1951). 5.00 gm. KI (A.R.) and 3.22 gm. KIO_3 (A.R.) were dissolved in 37.5 ml. distilled water, and to the solution was added 37.5 ml. conc. HCl and 5 ml. CCl_4. The mixture was then shaken vigorously for several minutes, thereby promoting the following reaction:

$$2I + HIO_3 + 3HCl \longrightarrow 3ICl + 3H_2O.$$

If the carbon tetrachloride layer did not have a faint pink colour, small amounts of KI were added until the presence of a little iodine was observed in the carbon tetrachloride. If the colour in the carbon tetrachloride layer was more than a little pink, some potassium iodate solution was added to convert a little of the iodine into iodine monochloride. This stock solution contained approximately 147 mg. of iodine per ml. as iodine monochloride. Aqueous solutions of iodine monochloride are stable for

several months in the presence of a high concentration of chloride ions and a slightly acid medium. Solutions of 0.42 mg. of I as ICl were obtained by 1 in 330 dilution of the stock iodine monochloride with saline. Such solutions were freshly prepared for each labelling.

(b) Preparation of Buffer Solutions

Two glycine buffers were used in conjunction with the labelling process, one, buffer B, for solution of the protein (pH 9 - 9.5), while the other, buffer A was used for the conversion of iodine monochloride to hypoiodite (pH 8.5).

Buffer A:- 9 ml. M-glycine in M/4 NaCl + 1 ml. N-NaOH.

Buffer B:- 8 ml. M-glycine in M/4 NaCl + 2 ml. N-NaOH.

(c) Preparation of Protein Solutions

3 ml. of glycine buffer B was mixed with 10 ml. of a 2% solution of the protein to be labelled.

(d) Iodination of Proteins

From a freshly prepared solution of iodine monochloride, (containing 0.42 mg. I/ml. 1.5 ml. was measured out. To this was added a solution (usually 5 ml.) of 5 millicuries of "carrier" free radioiodine (as $Na^{131}I$ or $Na^{125}I$, thiosulphate-free). Following addition of radioiodine, the iodine monochloride solution was converted to hypoiodite by addition of 3 ml. glycine buffer A. This solution was then immediately mixed with the protein solution and the labelled preparation transferred to a dialysis sac containing 1 gm. of "carrier" protein (bovine serum albumin). "Carrier" protein is added to reduce the specific activity of the preparation

to less than 5 μC/mg. protein, thereby reducing the possibility of radiation decomposition of the protein. The labelled protein was dialysed for 48 hours at 5°C against two 20 litre changes of 0.9% NaCl. The protein was then removed from the sac and centrifuged for 30 minutes at 1500 r.p.m. The radioactivity of such preparations was always more than 99% precipitable with T.C.A.

(e) Calculation of Iodine Incorporation into Albumin

Weight of Albumin labelled = 200 mgm.

Molecular Weight Albumin = 70,000 approx.

No. Molecules labelled = $\frac{6 \times 10^{23} \times 200}{7 \times 10^{4}} = 1.7 \times 10^{18}$.

1.5 ml. iodine monochloride contains 0.63 mgm. iodine.

i.e. $\frac{6 \times 10^{23} \times 0.63}{131 \times 10^{3}}$ atoms of iodine

$= 1.5 \times 10^{18}$ atoms.

Thus, assuming 100% incorporation, 0.9 atoms of iodine are incorporated per molecule of albumin.

Labelling of gammaglobulin, with its higher molecular weight, results in the incorporation of 2 atoms per molecule, using the same volume of stock iodine monochloride as above.

(xii) Labelling of Albumin with ^{95}Niobium

Albumin was labelled essentially by a modification of the method first described by Jeejeebhoy, Singh, Mani and Sanjana (1965) in which a buffered solution of human albumin was labelled with ^{95}Nb as niobium chloride. Carrier-free ^{95}Nb is obtained as an oxalate complex from the Radiochemical Centre, Amersham. This complex was decomposed and

converted to the chloride form for the labelling of albumin.

(a) Preparation of Niobium Chloride

1.5 ml. ^{95}Nb oxalate (containing 1.2 mc) was diluted to a volume of 5 ml. with distilled water and the solution evaporated to dryness. The solid was then dissolved in 5 ml. N/100 HCl, evaporated to about 2 ml., and finally diluted to 5 ml. by the addition of distilled water.

(b) Preparation and labelling of Protein Solution

600 mg. (4% solution) of rabbit serum albumin, prepared by molecular sieve chromatography was mixed with 10 ml. glycine buffer B and 2 ml. N/100 NaOH. This solution (pH 8) was then incubated at 37°C for labelling with ^{95}Nb chloride. The ^{95}Nb chloride solution, prepared as described was rapidly jet-sprayed into the buffered albumin solution, and the mixture (pH 7) incubated for 30 minutes at 37°C.

(c) Removal of Unbound ^{95}Nb

Unbound niobium was removed by passing the labelled protein solution through an alumina column (10 x 0.5 cm.) prepared in the hydroxyl form. N/100 NaOH was used to elute the protein from the column.

(d) Efficiency of Labelling

The total radioactivity of the effluent was calculated from the activity of a 1 ml. aliquot diluted to 5 ml. for counting. The activity retained on the column was determined by counting the alumina suspended in distilled water to volumes of 5 ml. 20% of the total activity added to the column remained bound to it, and of the activity associated with the effluent, 73% was protein bound as determined by T.C.A. precipitation.

The overall efficiency of labelling was thus calculated as being in the region of 55%. This compares favourably with the results of Jeejeebhoy et al (1965), whose labelling efficiency varied from 30 - 60% using human serum albumin. The labelled albumin was dialysed overnight against 20 litres of 0.9% NaCl, a procedure which did not increase the % activity precipitable with T.C.A. Prior to injection, the preparation was centrifuged for 30 minutes at 1500 r.p.m. No detectable precipitate was present

(xiii) Labelling of Erythrocytes with ^{51}Cr as Sodium Chromate ($Na_2{}^{51}CrO_4$)

Erythrocytes were labelled with ^{51}Cr by the method of Gray and Sterling (1950(a)). Incubation of red cells with ^{51}Cr as anionic hexavalent sodium chromate, results in the attachment of the label to the haemoglobin, and in particular to the globin portion of the molecule. In their original studies, Gray and Sterling (1950(a)) demonstrated that cationic trivalent chromic chloride ($^{51}CrCl_3$), which does not label intact erythrocytes is more efficiently incorporated into haemoglobin than the anionic hexavalent form. It is therefore considered that anionic hexavalent ^{51}Cr diffused through the red cell membrane, and upon reduction to the cationic trivalent state within the red cell, becomes bound to the haemoglobin.

(a) Procedure

Heparinised samples of blood were treated with ^{51}Cr as sodium chromate and the mixture incubated at 37°C for 1 hour with frequent mixing. The uptake of ^{51}Cr by red cells is rapid (Mollison and Veall, 1955) and a

one hour incubation period allows maximum uptake of the label. The amount of activity added was adjusted to yield a net activity of 100 μC. per ml. of packed red cells, assuming 50% incorporation of the label. Labelled blood samples were then spun for 20 minutes at 1500 r.p.m., the plasma removed and discarded and the cells washed with saline until removal of unbound ^{51}Cr was complete (Usually three such washings were sufficient). The labelled cells were then reconstituted approximately in proportion to the original haematocrit before injection.

(xiv) Radioactivity Measurements.

Radioactivity determinations were carried out in a well-type scintillation counter (Ekco Electronics Ltd.). Samples of blood and plasma were pipetted into counting tubes, and made up to a volume of 5 ml. with 0.01 N NaOH. Standard solutions were assayed for radioactivity and corrections for radioactive decay were based on the values for these solutions. Counts of less than three times background were considered to be beyond the lower limit for accurate determination.

SECTION I

PLASMA PROTEIN METABOLISM IN NORMAL AND FLUKE-INFECTED RABBITS

INTRODUCTION

The chemical composition of the blood of animals infected by F. hepatica has been studied by many workers. There is general agreement that an alteration of the plasma protein state occurs, this usually taking the form of a depression of albumin relative to globulin. Thus low albumin and increased globulin levels have been reported in rabbits (Secretan and Bickel, 1960), sheep (Ibrovic and Gall-Palla 1959; Nikolic, Nikolic and Nevenic 1962; Sinclair 1962 and 1968; Ross 1967; Ross, Dow and Todd 1967, Furmaga and Grundlach 1967; Jennings, Watts and Armour 1968), in cattle Nikolic et al. 1962, Hankiewicz 1965; Ross, Todd and Dow 1967) and in rats (Thorpe 1968). These changes in serum proteins are similar in all host species and first appear soon after the immature flukes reach the liver.

Determinations of certain enzymes in the blood and dye excretion tests have been used by some workers to assess the degree of tissue damage and impairment of liver function in fluke-infected animals. Urquhart (1955) in rabbits and Thorpe (1963) using rats could detect no increase in serum alkaline phosphatase in infected animals, while Jennings, et al. (1968) noted marked elevations in fluke-infected sheep only during the migratory phase of the disease. Vulcarenghi and Molinari (1959) failed to find elevated levels of serum glutamic oxalacetic transaminase (S.G.O.T.) in infected cattle, but Thorpe (1965), Ross et al (1966), Sinclair (1966), Jennings et al. (1968) reported significant increases. These latter authors noted however that increased levels only occurredduring migration,

and returned to normal soon after the flukes had become established within the bile-ducts of the host.

Bromsulphthalein (B.S.P.) excretion tests have also been used to assess liver damage in infected rabbits (Urquhart 1955), cattle (Cornelius, Theilen and Rhode 1958; Sewell 1966) and sheep (Sinclair 1966; Roberts 1968) with varying success. Urquhart (1955) found no retention of the dye in fluke-infected rabbits, while most other authors have noted impaired excretion, but this has only been marked when large numbers of flukes have been present.

While these studies have demonstrated significant changes in blood chemistry and liver function of animals infected with F. hepatica, they yield little information on the dynamic aspects of the disease. The plasma proteins are not merely confined to the circulation, but are extensively distributed in extravascular "compartments". Furthermore, they are in a state of flux with degradation and synthesis continuously replacing the population of protein molecules existing at any time. Until recently however the tendency has been to think in terms of changes in plasma concentration rather than of overall alterations in protein mass and turnover.

A change in plasma protein concentration does not necessarily reflect a change in the total mass of protein. For instance, a study on the aetiology of the hypoalbuminaemia associated with Type II ostertagiasis revealed that although the infected animals had lower serum albumin concentrations, the total mass of albumin present in the plasma did not

differ significantly from that of normal animals because an increased plasma volume had the effect of "diluting" the albumin present in the circulation, (Halliday, Mulligan and Dalton, 1968). On the other hand, a low plasma concentration may occur in oedematous conditions such as ascitis and pleural effusion as a result of abnormal distribution of protein between the intra- and extravascular pools (Berson and Yalow, 1959).

Thus in order to fully appreciate the protein status of the animal it is necessary to measure the size of the body pools of the protein under study and its distribution between them.

Although hypoproteinaemia may result from a variety of causes, the basic influence is disturbance of the equilibrium normally existing between synthesis and catabolism. For instance, it may result from subnormal synthesis which in turn may be due to an inadequate supply of the necessary amino acids, or to an impaired ability of the liver to synthesise proteins as occurs in chronic liver disease (Bauer, Blahd, Fields and Getchell 1954). Allbright, Bartter and Forbes (1949) first demonstrated that low serum protein levels may also be caused by excessive catabolism. These authors noted that some patients although suffering from an often severe hypoalbuminaemia produced and degraded more albumin than normal persons. The aetiology of such hypoproteinaemias had previously been considered to be impaired liver function, but it was clear that in such cases, instead of a defective production, an increased loss was responsible. Chronic loss of protein via the kidneys had previously been shown to account

for the substantial reduction in the plasma protein pool in nephrosis and other renal disorders (Blahd, Fields and Goldman 1955; Gitlin, Janeway and Farr 1956) but it was several years later before it was realised that loss of plasma proteins into the gastrointestinal tract could be of sufficient magnitude to account for the hypoproteinaemia associated with many diseases in man. These studies have been reviewed by Jarnum (1962).

It is impossible to assess the relative importance of subnormal synthesis and increased catabolism or loss as being the primary influence in determining the serum level of any protein without resorting to isotope techniques. It is only by the use of such techniques that accurate information on the amount of protein present, its distribution between the various body compartments and the rate at which it is lost from the available pool by catabolism may be obtained.

In the last decade in vitro labelling of proteins with radioactive iodine has been used extensively in man to study the behaviour of plasma proteins in vivo but apart from a few noteable exceptions these techniques have not been applied to animals suffering from parasitic diseases. Smithers and Walker (1961) first measured the turnover of ^{131}I-labelled albumin in monkeys infected with Schistosoma mansoni, while Mulligan, Dalton and Anderson (1963) and Nielsen (1966) used this label to elucidate the mechanism of the hypoalbuminaemia associated with bovine ostertagiasis.

Nielsen and Nansen (1967) have studied IgG immunoglobulin turnover in diarrhoeal disorders of cattle and swine but despite their obvious

importance and relevance to parasitic infections, no other studies appear to have been published.

The cause of reduced albumin levels in chronic fascioliasis has not yet been completely determined, but there are basically two schools of thought on the problem. Some workers consider that because of the extensive damage to liver tissue during migration of the parasite to the bile ducts some interference with albumin synthesis is responsible (Thorpe 1965; Sinclair 1962, 1968), while Jennings et al (1956) consider that loss of plasma constituents via the bile due to the feeding activities of the flukes is the most likely cause.

In the work described in this section of the thesis, the turnover of albumin was studied with a view to determining whether subnormal synthesis or increased catabolism was mainly responsible for causing the hypoalbuminaemia associated with the disease. This was done by following the fate of intravenously injected homologous ^{131}I-labelled albumin in normal and fluke-infected rabbits. Because of the likelihood that increased catabolism of this protein could be due to its passage into the gut and subsequent digestion by intestinal enzymes, the daily faecal output of each rabbit was analysed for radioactivity.

Since faecal excretion of the isotope following injection of radio-iodinated proteins does not give a true estimate of enteric protein leak due to reabsorption following catabolism of the label, the faecal excretion of ^{131}I-labelled polyvinylpyrrolidone (P.V.P.) was studied in a second group of infected and control rabbits. This macromolecule is neither

degraded by intestinal enzymes nor absorbed from the gut on oral administration and is therefore a better quantitative test of abnormal passage of plasma macromolecules into the intestinal tract than radioiodinated protein. The P.V.P. test has been successfully used by Mulligan et al (1963) in cattle suffering from ostertagiasis.

In order to study the aetiology of the high serum gammaglobulin concentrations usually found in infected animals, a simultaneous double-labelling experiment was carried out in which albumin was trace-labelled with ^{125}I and 7S-gammaglobulin (the most abundant class of immunoglobulin) with ^{131}I. These were injected into normal and fluke-infected rabbits. It was intended that these studies would indicate whether gammaglobulin was being catabolised at a similar rate to albumin in fluke-infected animals, and if this was the case, certain inferences could then be drawn about the relative rates of synthesis of these two proteins under normal and pathological conditions.

The section begins however by considering the assumptions which must be made about the labelled preparation, the animals under study, and the sites of synthesis and catabolism of plasma proteins before data obtained from the use of radioiodinated plasma proteins may be properly analysed. The precautions which must be taken to minimise damage to protein molecules in the labelling process are discussed since it must be assumed that the labelled preparation behaves in the same way as unlabelled molecules. The sites of synthesis and catabolism and the labels currently

used for detecting and quantitating passage of proteins into the intestinal tract are also considered since these have an important bearing on the validity of the assumptions upon which the mathematical model used for analysis of the data are based.

(1) Assumptions about the Labelled Material

It is of course fundamental that the metabolic behaviour of the labelled protein is characteristic of the substance under study, i.e. that the animal should not be able to distinguish between genuine and labelled molecules. In addition, there should be no reutilisation of the label for synthesis of new protein after the original material is degraded and the label, once liberated by protein breakdown should be rapidly and completely excreted.

Two factors are important in determining the animal's ability to distinguish between its own and injected radioiodinated molecules - the methods of protein isolation and labelling. The proteins used in these studies were prepared by isolation procedures likely to cause minimal alterations to protein structure. Three main precautions must be taken when labelling plasma proteins to avoid denaturation. In the first place, the protein should not be over iodinated otherwise the preparation will not behave in the same way as unlabelled molecules in a biological test (Berson, Yalow, Schreiber and Post 1953). However it should be noted in this respect, that proteins vary greatly in their sensitivity to iodination. For example, incorporation of 2 -3 atoms per molecule into albumin has no

deleterious effect, whereas transferrin, caeruloplasmin, haptoglobin, fibrinogen and gamma $_M$ globulin show evidence of denaturation if a mean level of 0.5 atoms per molecule is exceeded. Gamma $_G$ globulin by contrast can be iodinated at 1.5 atoms per molecule with no apparent denaturation. Secondly, the protein should as far as possible be evenly iodinated and this is best achieved by the jet-iodination method of McFarlane (1958) using iodine monochloride as "carrier". Thirdly, care should be taken to minimise "radiation-decomposition". This results from the self-irradiation of the protein by its label causing abnormal metabolic (Berson et al 1953, Freeman 1959) and chromatographic (Fahey, McCoy and Goulian 1958; Cohen 1959) behaviour. It is necessary to reduce the specific activity of the preparation to about 5 μc/mg. in order to avoid the possibility of radiation damage (Berson et al 1953) especially if the labelled preparation has to be stored for any length of time prior to use (Cohen 1959).

Proteins isolated and iodinated with attention to the details described above behave like the native proteins. Thus it has been shown that ^{131}I-labelled albumin and globulin behave in the same was as the ^{14}C-labelled proteins in the rabbit (Cohen, Holloway, Matthews and McFarlane, 1956). In man, it was also found that in a case of analbuminaemia, following intravenous injection of unlabelled albumin and a preparation labelled by McFarlane's method, the two preparations disappeared from the body at the same rate.

It is important to realise that labelled protein may be identical with non-labelled molecules by in vitro tests e.g. electrophoresis and

ultracentrifugal analysis, but may nevertheless not behave in the same way *in vivo*. Some form of *in vivo* testing must therefore be used. High urinary excretion of radioactivity in the first few days of a study indicates denaturation of a labelled protein. This is because denatured protein is rapidly taken up by the reticuloendothelial cells especially in the liver, the protein is degraded and the label is rapidly excreted in the urine.

Use is made of this rapid removal of denatured protein by a biological "screening" test (McFarlane 1956). The labelled material is injected into an animal, and a few days later some of its plasma transferred to a second animal. The metabolic study can then be carried out using a protein uncontaminated by denatured molecules. Since every precaution was taken in the isolation and labelling of proteins used in these studies, it was considered that the results obtained would be an accurate reflection of the turnover of the protein concerned.

The validity of the assumption that no reutilisation of the isotope occurs following metabolic breakdown of radioiodinated proteins has been clearly demonstrated. Following an oral dose of ^{131}I-labelled albumin, the label is not incorporated into protein molecules (Cohen *et al*, 1956; Zizza, Campbell and Reeve 1959), nor is the body able to reutilise radioiodine-labelled tyrosine residues following oral or intravenous injection (McFarlane 1957, Zizza *et al* 1959). Thus radioiodinated proteins do not suffer from the drawback of reutilisation of the label following their degradation. Assuming that the labelled protein behaves metabolically like non-labelled molecules and that the label once released by catabolism

is not reutilised for further protein synthesis, it can be safely assumed that the excreted radioactivity is derived only from breakdown of the protein. Before excretion, the iodine released by protein catabolism is distributed throughout the iodide pools of the blood, tissue fluids and thyroid gland. Since in the rabbit, these pools have a combined volume of roughly 8 times that of the plasma iodide pool (Zizza et al 1959), there is a time lag of several hours before iodide released by albumin catabolism is distributed throughout the iodide pools and excreted. Thus during the first day or so following injection of radioiodinated proteins, the activity excreted will be an underestimate of protein catabolism, unless corrected for body water radioactivities. However after the initial "charging-up" of the iodide pools the delay in excretion of the isotope following protein catabolism will be of minor importance, provided the thyroid is "blocked" by prior administration of inactive iodide. Excretion of the label will then be practically identical to its release following protein degradation.

It is apparent that there will always be a small amount of radioactivity which is not associated with the labelled protein. When the rate of catabolism of the protein under study is low and provided renal function is normal, this usually forms an insignificant proportion of the total plasma (and extravascular)activity, and the delay in iodide excretion produced by the iodide pool will therefore by small. When rapidly catabolised proteins are being studied or when the animal has renal failure, a significant amount of the activity retained in the body is represented by iodide in the body water and it is necessary under these circumstances

to take the iodide pool into account. Provided the thyroid is "blocked" it can normally be assumed that the label once released is rapidly and quantitatively excreted. The main route of excretion is the kidney, but passage of labelled iodide also occurs into the gastro-intestinal tract. Faecal excretion however is normally negligible due to reabsorption of iodide by the stomach and small intestine (Parkins, Dimitriadou and Booth, 1960).

(2) Assumptions about the Sites of Synthesis and Catabolism of Plasma Proteins

(a) Plasma Protein Synthesis

Plasma albumin and most of the alpha and beta - globulins are synthesised in the liver. Hepatectomised animals are unable to synthesise albumin (Roberts and Brumish 1953) whereas liver tissue incorporates labelled amino acids into albumin, alpha and beta globulin (Peters and Alfinsen 1950; Miller and Bale 1954). Peters (1959) showed the actual site of albumin production to be the microsomes of the parenchymal cells, and this author has since succeeded in extracting from these structures immuno-electrophoretically identifiable albumin (Peters 1963). A decrease in serum albumin concentration is a common finding in disease conditions associated with liver injury due to diminished hepaticcapacity to synthesise this protein and subnormal synthesis has therefore been assumed by some workers (Thorpe 1965; Sinclair 1962, 1968) to be responsible for the hypo-albuminaemia associated with chronic fascioliasis.

At the moment no information is available as to the cause of the increased gamma globulin levels in fluke-infected animals. The term "serum

gammaglobulin" is rather non-specific in view of the heterogeneity of this protein group. Ultracentrifugal analysis has shown that two broad classes of gammaglobulin exist - 19S (large molecular weight macroglobulins, also known as IgM), and the more abundant 7S group (molecular weight 160,000, and known as IgG). A further class IgA with an electrophoretic mobility like IgM (i.e. that of a beta-globulin) has also been recently found.

Gammaglobulins are produced by the plasma cells and related cells of the reticuloendothelial system, for example lymphocytes. It has been consistently shown that serum gammaglobulin levels are related to the number of plasma cells present. Thus hypogammaglobulinaemia is associated with those malignant diseases which tend to reduce the amount of normal reticuloendothelial cells, e.g. lymphatic leukaemia and malignant lymphoma (Fairley and Bodley Scott 1961; Linton, Dunning and Thomson 1963). The low serum gammaglobulin levels frequently seen in these disorders is due to subnormal synthesis rather than increased catabolism (Andersen 1964). Hypogammaglobulinaemia also results from irradiation and the administration of adrenal cortical hormones (Talmage 1956; Benacerraf 1960; Bjorneboe, Fischel and Stoerk 1951; Berglund 1956). These have the effect of markedly reducing the amount of lymphatic tissue. A number of diseases in man are associated with increased levels of gammaglobulin in the serum. e.g. Chronic liver injury, collagen disease (Jarrold and Vilter 1949; Mills, Calkins and Cohen 1961). The degree of hypergammaglobulinaemia produced in these conditions is likewise related to the number of gammaglobulin producing cells (Ghagov, Kent and Popper 1959; Cohen, Ohta, Singer and Popper 1960).

It is interesting to note that after certain types of antigenic stimuli, the first reaction is increased systhesis of IgM, which is relatively soon followed by an increase in IgG synthesis (Lospalluto, Miller, Dorward and Frisk 1962; Bauer, Mathies and Stavitsky 1963; Uhr and Finkelstein 1963). Recent evidence on the formation of immunoglobulins by human tissue in vitro has further indicated that the lymphocytes are responsible for IgM antibody production (R. van Furth 1964). The immune reaction may therefore involve a primary antigenic stimulus provoking a production of IgM globulin antibody in the lymphocytes which are then transformed to plasma cells with subsequent production of IgG antibody.

Little is known about the production of IgA except that it is present in relatively high concentration in colostrum, saliva and bronchial secretions. Plasma cells synthesising IgA antibodies are present in large numbers in the lamina propria of the intestinal tract, and this local production of antibody is possibly of importance in the elimination of intestinal parasites as a result of an immune response on the part of the host (Mulligan 1963).

The anatomical sites of plasma protein synthesis are such that synthesis can be considered from a functional viewpoint to be intravascular. Newly synthesised albumin on leaving the liver cells enters either the venous blood or the hepatic lymph. The fact that labelled albumin is present in the bloodstream within 2 hours following intravenous injection of ^{35}S methionine (Tarver and Reinhardt 1947), indicates that by either route it will enter the circulation almost as soon as it is released from the parenchymal cells.

Since the lymphatic drainage of bone marrow, spleen and thymus is insignificant, gammaglobulins produced in these organs will be immediately discharged into the blood following their release from plasma cells. Those produced in the liver and lymph nodes will probably be removed mainly by the plasma, but partly also by the lymphatics. However lymph flow to the bloodstream is rapid and it can therefore be reasonably assumed that gammaglobulin synthesis, like that of albumin is intravascular.

(b) Catabolism of Plasma Proteins and the Measurement of Gastro-Intestinal Leak under Normal and Pathological Conditions

At present the main site of catabolism of plasma proteins is not known, although nearly every organ in the body has at one time or in one condition been shown to effect the release of serum protein degradation products. Early studies indicated that they were universally degraded by conversion to cell protein. Gitlin, Landing and Whipple (1953) showed by use of the fluorescent antibody technique that albumin, gammaglobulin and transferrin were present intracellularly, and Yuile, Lamson, Miller and Whipple (1951) after intravenously injecting ^{14}C-labelled plasma proteins found 30 - 40% of the label to be associated with extravascular protein. No distinction however was made between interstitial and intracellular uptake. Recent evidence now suggests that plasma proteins do not play any significant part in tissue protein synthesis. Eagle and Piez (1960) for example found insignificant cellular utilisation of plasma proteins in cultures of malignant human tissue.

Although the site is not precisely known, indirect evidence obtained by the use of radioiodinated plasma proteins indicates that it must be in

close proximity, at least from a functional point of view, to the plasma. Since catabolism of an iodine-labelled protein results in excretion of its radioactive breakdown products, the activity excreted during each day following injection should be proportional to that present in the protein pool in which catabolism is taking place. Only the assumption of intravascular catabolism of albumin and gammaglobulin fulfills the requirement of a constant degradation from day to day of these proteins (Campbell et al 1956; Freeman and Matthews 1958; Reeve and Roberts 1959; Cohen and Freeman 1960; McFarlane 1963 (a)).

Further evidence of an essentially "intravascular" catabolism is also indicated from the fact that the protein-bound urine activity of nephrotics makes up a constant fraction of the total plasma activity (Pearson, Veall and Vetter 1958). This is only possible when degradation is limited or very nearly so to circulating albumin.

Since iodine-labelled plasma proteins are not significantly broken down by incubation with plasma or blood in vitro at body temperature, they must come in contact with cells containing the essential proteolytic enzymes. Cohen and Gordon (1958) using perfused rat livers and biologically "screened" preparations of labelled albumin showed that this organ may normally be responsible for about 15% of the total catabolism of albumin. This is in contrast to its marked ability to remove heat-denatured protein and particulate material from the circulation (Gordon 1957). By blocking the Kupffer cells with carbon particles, it has further been shown that hepatic degradation of undenatured molecules does not occur in the reticulo-

endothelial component of the liver (Freeman, Gordon and Humphrey 1958).

The above findings are at variance with those of Gitlin, Klinenberg and Hughes(1958). Using an unscreened commercial radioiodinated albumin these authors found a marked decrease in the rate of disappearance of the label from the circulation of hepatectomised mice, with an additional decrease following blockage of the Kupffer cells with thorium dioxide and Indian ink. However these results do not give a reliable indication of the normal role of the liver in protein catabolism since the labelled preparation probably contained significant amounts of heavily denatured protein. Furthermore, since the plasma disappearance curve is as much a reflection of the rate at which newly synthesised molecules enter the circulation as it is of their removal from it by catabolism, removal of the organ in which albumin is synthesised will have the effect of decreasing the rate of disappearance of the labelled protein from the circulation.

The liver has similarly been shown to be responsible for about 30% of the total gammaglobulin breakdown (Cohen, Gordon and Matthews 1962). Since it is probable that gammaglobulins are partly broken down in the reticuloendothelial cells following reactions with antigens (Benacerraf, Sebestyn and Cooper 1959), the fact that the liver contains such cells explains its role as a major site of gammaglobulin catabolism.

The kidney has been proposed as a degradation site of plasma proteins by virtue of the constant filtration and reabsorption of plasma constituents by the proximal tubule cells (Sellers 1956). The possibility of this organ being responsible for plasma protein catabolism has been studied in the nephrectomised rat and in the isolated perfused rabbit kidney

using homologous ^{131}I-labelled plasma albumin (Katz, Rosenfield and Sellers 1960). These authors showed that, as in the case of the liver, albumin breakdown varied with the characteristics of the labelled protein used. Renal degradation was responsible for about 30% of the total catabolism of an "unscreened" preparation, whereas when a "screened" protein was used, the kidney only contributed in the region of 10% to the total degradation.

Increased catabolism of plasma proteins by leakage into the gastro-intestinal tract and subsequent digestion by intestinal enzymes is the cause of the hypoproteinaemias associated with some intestinal disturbances in man e.g. regional enteritis, ulcerative colitis, hypertrophic gastritis (Steinfield, Davidson and Gordon, 1957; Citrin, Sterling and Halsted 1957).

The presence of albumin and gammaglobulin has been demonstrated in saliva, gastric and intestinal juice (Holman, Nickel and Sleisenger 1959; Armstrong, Margen and Tarver 1960; Campbell, Cuthbertson, Mackie, McFarlane, Phillipson and Sudsaneh 1961; Glenert, Jarnum and Reimar 1962; Andersen 1963).

These findings initiated research into the preparation of labels suitable for detecting and quantitating the passage of plasma proteins into the gastro-intestinal tract under normal and pathological conditions.

The ideal label for this purpose should have certain characteristics. It should be firmly attached to the protein under study without altering its biological properties. If this were achieved, the labelled protein could then be used to quantitate simultaneously the rates of intestinal protein loss and normal endogenous catabolism. In addition, it is essential that the label on entering the alimentary tract should not be

reabsorbed, otherwise the extent of the intestinal protein loss would be underestimated. No loss of label into the gut should occur in any form other than as the intact labelled protein, since this would lead to over-estimation of the intestinal protein leak.

The following preparations are employed for direct estimation of the loss of macromolecular substances into the intestinal tract.

(3) Radioiodinated Plasma Proteins

Although radioiodinated proteins may be used to determine the rate of protein catabolism (and synthesis in the "steady state") they cannot normally be used to quantitate gastro-intestinal loss because of reabsorption of the isotope following degradation of the protein in the alimentary tract. Therefore protein loss cannot be quantitated by determining faecal radio-activity.

In an attempt to overcome the problem of radioiodide absorption from the gut, Jeejeebhoy and Coghill (1961) gave an ion exchange resin orally in conjunction with the intravenously injected labelled protein, the idea being that iodide released by protein catabolism would become bound to the resin and thus be quantitatively excreted in the faeces. However it was subsequently shown that iodide liberated by degradation of protein anywhere in the body can pass into the gut via the saliva and intestinal secretions and thereby become attached to the resin (Jones and Morgan 1963; Freeman and Gordon 1963; Chapman, Jeffries and Sleisenger 1964). Thus faecal radioiodine excretion in subjects with normal albumin catabolism bears no quantitative relationship to the enteric leak of labelled albumin.

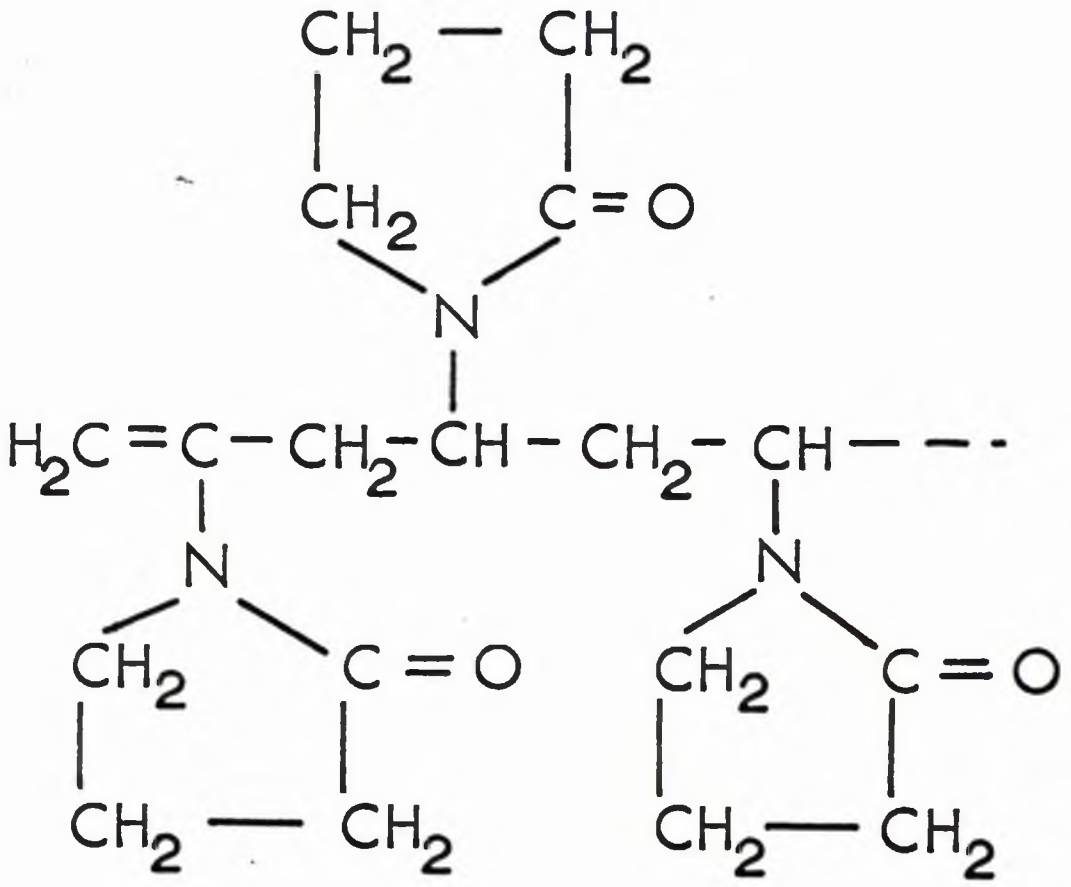
An elevated faecal radioiodide excretion is regularly found in cases of protein loss into the stomach and small intestine (Steinfield et al 1957; Jarnum 1961; Schwartz and Jarnum 1961). Nielsen (1966 (a) and (b)) was also able to demonstrate faecal excretion of plasma protein in cattle infected with Ostertagia ostertagi, and in pigs with gastro-intestinal diseases. The appearance of excessive amounts of the label in the faeces is thus a good qualitative test of protein loss into the alimentary tract. It should however be noted in this respect that except in those instances of excessive loss into the lower bowel, diagnosis can be obscured by intestinal absorption of nearly all the isotope before it is excreted in the faeces.

(4) ^{131}I-labelled Polyvinylpyrrolidone (P.V.P. - Fig. 4)

The introduction of ^{131}I-labelled P.V.P. by Gordon (1959) has enabled a more accurate assessment of the magnitude of macromolecular loss into the gastro-intestinal tract since it is neither degraded nor absorbed on oral administration. Radioiodinated P.V.P. obtained from the Radiochemical Centre, Amersham, has an average molecular weight of about 40,000 (Range 10,000 - 90,000) and although chemically, physically and biologically very different from serum proteins, the faecal excretion of the label gives a good indication of the degree of macromolecular leakage. It was through the use of this substance that widespread recognition was given to the concept of protein-losing gastroenteropathy as being the cause of many hypoproteinaemias of previously unknown aetiology (Jarnum 1962). In the field of veterinary medicine, Mulligan, Dalton and Anderson (1963) first used ^{131}I-labelled P.V.P. to demonstrate abnormal intestinal protein loss

FIGURE 4

POLYVINYLPYRROLIDONE



as being the cause of the hypoproteinaemia associated with Ostertagiasis in cattle.

The main disadvantages of P.V.P. are that it is not a natural substance and has a spectrum of molecular weight. It is therefore not possible to convert results obtained in terms of P.V.P. excretion into loss of albumin or any other plasma protein although a good general correlation between increased faecal excretion of the label and inferred increased losses of plasma proteins into the gut exists.

(5) ^{95}Nb-Labelled Albumin

Albumin labelled *in vitro* with ^{95}Nb was first used by Jeejeebhoy, Singh, Mani, and Sanjana (1964) as a plasma protein tag. Although the label fulfills the requirement of not being reabsorbed from the gut on oral administration and can therefore be used to detect and probably quantitate gastro-intestinal protein leak, it cannot be used to simultaneously estimate albumin turnover due to partial denaturation of the protein during the labelling procedure. Following intravenous injection the label disappears rapidly from the circulation for 2 to 3 days, and thereafter exponentially although at a slightly more rapid rate than radioiodinated albumin. Experience of labelling rabbit albumin with ^{95}Nb has shown that significant amounts of non-protein bound ^{95}Nb are associated with the preparation. Niobium is also known to form colloids and the initial rapid decline in plasma activity following injection may therefore be due to uptake of these colloids by the reticuloendothelial system, in addition to rapid renal removal of unbound ^{95}Nb. These findings are supported by Matthews and Garteide (1965)

who studied organ uptake of ^{95}Nb in rats. They showed that although the liver/plasma activity ratio was initially high it decreased with time, while the activity in the kidney and spleen remained practically constant. This may be either due to release of ^{95}Nb after protein catabolism in a form which is taken up by these organs, or alternatively that albumin is catabolised by them.

Systematic studies on the durability of ^{95}Nb-labelled albumin have not been carried out and it is assumed that the label remains firmly bound to albumin. Studies with the label on normal and fluke-infected rabbits have shown a good correlation between the degree of anaemia observed and faecal excretion of the isotope, while normal animals excrete only a small fraction of the injected dose (Dargie Sections 3 and 4). Similar results have been reported in humans suffering from various gastro-intestinal disorders (Jeejeebhoy, Jarnum, Singh, Nadkarni and Westergaard 1968).

(6) ^{51}Cr-Labelled Albumin

Because of its property of readily binding to proteins, cationic trivalent chromic chloride has been used to label plasma proteins. Following intravenous injection of $^{51}CrCl_3$ in man, much of the label binds to the alpha$_2$ globulins, and only about 20% becomes attached to albumin (Guillen and Peterson 1964).

Albumin labelled with ^{51}Cr *in vitro* was introduced by Waldmann (1961) for the study of protein-losing gastroenteropathy. The rate of disappearance of this preparation from the circulation is much shorter than that of ^{131}I-labelled albumin and it is therefore not generally useful as an indicator of albumin catabolism. The isotope is not firmly attached to the albumin

since much of it becomes associated with alpha$_2$ globulins following injection (Cuillen and Peterson 1964). Thus the plasma disappearance curve after intravenous administration of ^{51}Cr, either as $^{51}CrCl_3$ or ^{51}Cr-labelled albumin is probably more an indicator of transferrin than albumin degradation (Van Tongern and Reichert 1966).

However it has been adequately demonstrated that no significant reabsorption of ^{51}Cr occurs from the alimentary tract of man, dog and rabbit Perez-Gimiez, Layrisse, di Prisco 1957; Clark, Kling, Woodley and Sharp 1961; Jennings 1962), although in sheep a small amount of the label can be absorbed into the circulation (Clark, Kiessel, Goby 1962). Since the label in most cases is quantitatively excreted in the faeces after oral administration, it provides a useful tool for studying protein loss into the alimentary tract. Moreover it is particularly useful when used in conjunction with ^{131}I-labelled albumin allowing simultaneous measurement of catabolic rate and intestinal protein leak. A good correlation between increased catabolic rate of albumin and the magnitude of its leak into the intestinal tract has been shown in patients suffering from various gastro-intestinal disorders (Waldmann and Wochner 1964) and in sheep infected with F. hepatica (Holmes 1969).

It is apparent that none of the above preparations is ideal for quantitating intestinal protein loss although all are capable of detecting it. Recently however ^{67}Cu-labelled caeruloplasmin has been successfully used to simultaneously measure the turnover and intestinal leak of this protein (Waldmann, Morell; Wochner, Strober and Sternlieb 1967). The

labelling procedure involves exchanging radio-copper with the copper atoms of caeruloplasmin *in vitro* the unbound isotope being subsequently removed by passing the labelled preparation through an iron-exchange resin. This preparation behaved in an identical fashion to ^{131}I-labelled caeruloplasmin and could therefore be used for turnover studies. In addition, the radio-copper moiety was poorly absorbed from the gut and was not actively secreted into it (Aisen, Morell, Alpert and Sternlieb 1964). ^{67}Cu-labelled caeruloplasmin would therefore appear to fulfill all the requirements of a theoretically ideal label, but since the normal serum concentration of the protein is 20 - 35 mg. per 100 ml. (Sternlieb and Scheinberg 1961), the difficulties involved in its separation and the short half-life of ^{67}Cu (62 hours) make it less attractive than it first appears.

The magnitude of the normal leak of plasma proteins into the gut is at present the cause of much controversy. Some authors consider that intestinal leakage of albumin and gammaglobulin and subsequent digestion and reabsorption of the products, accounts for 50 - 70% of the total daily catabolism of these proteins while others believe that it represents less than 10% of the total.

Evidence for the former view is given by the results of studies using perfused or isolated loops of guts of rabbits (Armstrong *et al* 1960); sheep (Campbell *et al* 1961); dogs (Andersen 1963) and cattle and pigs (Dich and Nielsen 1964). These authors demonstrated sufficient labelled protein in the lumen of these segments following intravenous injection of the label to account for the major part of normal catabolism.

Since it is impossible to collect all the secretion from the whole intestinal tract it is difficult to estimate from their data the extent of leakage in the whole intestinal tract, especially when in many cases the results were derived from the upper physiologically more active part of the alimentary tract.

These findings are supported by the work of Benson, Kim and Bollman (1955) who showed that the specific activity of intestinal lymph after intravenous injection of ^{131}I-labelled albumin rapidly equals that of the plasma, indicating that considerable amounts of circulating plasma proteins must leave the blood through the gastro-intestinal capillaries each day. It has not yet been shown however whether most of this protein is recovered in the lymph and subsequently returned to the bloodstream and whether part of it is transferred to the lumen of the gut and stomach.

The mechanism of normal protein passage into the digestive tract isn't known - it may be secreted through the epithelial cells or more likely lost in the extrusion of epithelial cells into the gut. A third possibility is that they leak through a membrane which is not entirely protein tight. The findings of Murray (1968) are significant in this respect. Electron microscopic examination of intestinal tissue of cattle infected with ostertagiasis has shown the presence of electron-dense material lying in the intercellular spaces of cells lining the gastric mucosa. On the basis that the electron density of this material was similar to that of known protein- containing structures within cells such as zymogen granules, Murray concluded that it was protein. Since such a mechanism of plasma protein

transfer into the alimentary tract has not been reported in normal animals or man, it is most likely that under normal conditions plasma proteins pass into the lumen as a result of extrusion of intestinal cells.

Jeejeebhoy et al (1963) and Waldmann (1961) using ^{95}Nb and ^{51}Cr-labelled albumin respectively showed that only a small proportion of the injected dose could be found in the faeces, and indicated that less than 10% of the total daily catabolism of albumin in man could be attributed to intestinal leakage. Additional support for the hypothesis of a small plasma protein leak comes from the work of Franks, Mosser and Anstadt 1963. They have shown that removal of 70% of the jejunum and ileum does not reduce the rate of albumin catabolism in the rabbit. In addition, Jeejeebhoy and Coghill (1961) using ^{131}I-labelled albumin in conjunction with an oral ion-exchange resin calculated that only about 20% of normal albumin catabolism occurs in the gut, and Freeman and Gordon (1964), using the same method but taking into account salivary and intestinal radioiodide activity concluded that no more than very small amounts of albumin normally pass into the digestive tract.

Recent evidence against a large enteric leak of plasma proteins has been obtained through the use of ^{67}Cu-labelled caeruloplasmin (Waldmann et al 1967). These authors have shown that although caeruloplasmin is present in gastro-intestinal secretions (Wilson, Heiner and Lahey 1962), the amount which leaks into the intestine only accounts for 11% and 15% of the overall metabolism in dog and man respectively. Although this conclusion cannot be extended to serum proteins other than caeruloplasmin (Mol. wt. 151,000,)

Waldmann concluded that bulk loss of serum into the gut is not of major importance in normal protein catabolism.

Thus, although there is no doubt that plasma protein degradation does occur in the digestive tract, its quantitative importance remains to be settled.

However catabolism of albumin and gammaglobulin occurring in the liver, kidney or gut is compatible with evidence for an "intravascular" site, i.e. although not actually broken down in the bloodstream (Reeve and Roberts 1959), they are catabolised at sites in so rapid exchange with plasma that the specific activity of the protein at the degradation site is identical with that of the protein in the plasma. It may therefore be assumed that catabolism of the plasma proteins in addition to synthesis occurs intravascularly.

(7) Assumptions about the Animals under Study

The serum concentration of any protein is merely a reflection of the dynamic equilibrium that exists between catabolism and synthesis i.e. of the turnover of the protein. The magnitude of the turnover rate can only be determined from measurement of either one of these two processes if the animal is in a "steady state" with respect to the protein under study, i.e. if the rate of protein synthesis is equal to the rate of loss or degradation. In applying radioiodinated proteins, only the amount or fraction of the protein broken down per unit of time is measured, and at constant protein concentration and plasma volume, synthesis is indirectly evaluated.

For normal animals it can be safely assumed that synthesis and catabolism are in equilibrium, whereas in fluke-infected rabbits for example, this assumption may not be valid. Under these circumstances, although only degradation can be estimated, certain comparisons may still be drawn regarding synthesis between the two groups. For instance, if degradation of a particular protein was markedly increased, and the amount present in the plasma only marginally reduced, it would be reasonable to assume that in order to maintain this situation synthesis must be proceeding almost as fast as catabolism. Valuable information may therefore still be obtained under non steady-state conditions, provided the limitations are realised.

(3) The Protein "Pools" of the Body

The plasma proteins are not confined to the bloodstream but are distributed throughout the body in various "compartments" or "pools". By canulating lymphatics and injecting radioiodinated proteins it has been established that large amounts of protein leave the bloodstream and pass into the tissue interstitial spaces from which they enter the lymphatic circulation to be transported back into the circulating blood (Wasserman and Mayerson 1951). The overall permeability of organs to the plasma proteins varies greatly - liver, spleen and small intestine are highly permeable, while muscle and skin have low permeabilities (Benson et al 1955). These differences are due mainly to differences in the vascularity of the tissues (Dewey 1959), to variations in the number and size of capillary pores (Mayerson, Wolfram, Shirley and Wasserman 1960), and the presence, absence and type of basement membrane associated with the capillary endothelium (Bennett, Luft and Hampton 1959).

The size and shape of the protein also plays a major role in determining its ability to pass out of the circulation. It has been established that more than half of the total body pool of albumin, IgG, and transferrin (molecular weights 69,000, 160,000 and 90,000 respectively) is present extravascularly (Sterling 1951; Berson et al 1953; Cohen and Freeman 1960; Jarnum and Lassen 1961; Solomon, Waldmann and Fahey 1963). Noteable exceptions to this are IgM and fibrinogen (molecular weights 1 million and 340,000 respectively) which are mostly confined to the circulation (Cohen and Freeman 1960; Barth, Wochner, Waldmann and Fahey 1964; McFarlane 1963 (b)). These observations are compatible with a hypothesis that normal passage of proteins out of the vascular compartment involves transit through pores or other structures of a limiting size.

The extravascular distribution of albumin has been studied in man and in rats using ^{131}I-labelled albumin (Rothschild, Bauman, Yalow and Berson 1955; Sellers, Katz, Bonorris and Okuyama 1966). These authors have shown that there are at least two main groups of extravascular compartments which equilibrate with plasma at different rates. Most of the extravascular albumin could be accounted for in skin and muscle (about 60%) while the remainder was associated with the liver, heart, kidney, spleen and gastrointestinal tract. Berson et al (1953) injected ^{131}I-labelled albumin into skin and muscle and found that it took 4 - 5 days for complete equilibration with plasma. On the other hand, it is likely that the liver, gut, etc. have albumin pools which equilibrate with plasma albumin very rapidly since it has been established that hepatic and intestinal lymph specific activities

equal that of the plasma within 8 hours following injection of ^{131}I-labelled albumin (Berson et al 1955).

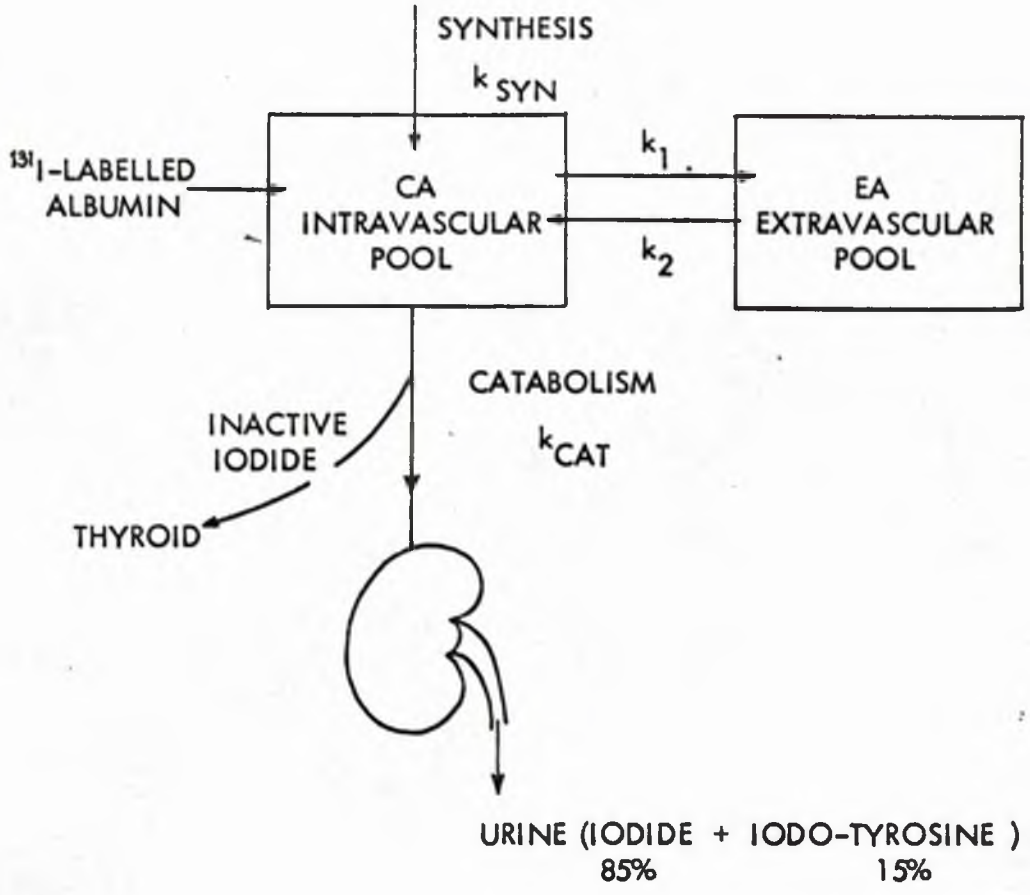
The finding of two main extravascular compartments is in good agreement with "indirect" evidence obtained from graphic analysis of plasma activity curves following injection of ^{131}I-labelled proteins. Thus the plasma disappearance curve is often reported to be the sum of three exponentials, indicating the presence of two extravascular pools (Berson et al 1953; Matthews 1957; Halliday, Mulligan and Dalton 1963; Dargie Section 1).

These results indicate that the total extravascular pool consists of several pools of different masses with different exchange rates from the plasma to each. It is therefore necessary to visualise in addition to a ramifying plasma pool an even more highly subdivided extravascular pool of similar proteins.

However such a multicompartment system is very complex and most mathematical models describing protein behaviour represent all the extravascular pools by one common pool communicating with the plasma through pores in the capillary wall, i.e. as a two-compartment system (Fig. 5). This system is "open" in that protein transfer from one pool to another can take place in either direction, k_1 reflecting the capillary permeability of the protein under study and k_2 the lymphatic flow rate. It should however be noted that since extravascular protein is localised in an infinite number of different compartments each of which has an individual exchange rate, k_2 represents an average of the return rates for the protein from all extravascular pools.

FIGURE 5

NORMAL DISTRIBUTION AND METABOLISM OF RADIOIODINATED PLASMA PROTEINS



All mathematical models used to determine turnover rates of labelled proteins assume that the animal is in a steady state i.e. that $k_{sym.}$ and $k_{cat.}$ are equal. A further requirement is that CA and EA remain constant, and it is clear that this can only prevail if k_1 / k_2 remains unaltered. Assuming a metabolic steady state, it is apparent that the rate of synthesis is dependent only on CA and $k_{cat.}$

i.e. Rate of synthesis = CA x $k_{cat.}$

In the studies reported in this thesis steady state conditions may only be reasonably assumed in the normal rabbits, but as previously indicated valid conclusions may still be made about the rate of protein synthesis in infected animals.

MATERIALS AND METHODS

(i) Experimental Animals

Four fluke-infected and four normal rabbits were used, each infected animal having received 100 metacercariae 8 - 9 weeks previously. Since flukes normally enter the bile ducts of the rabbit about 6 weeks post-infection (Urquhart 1955), it was considered that the infected rabbits were harbouring populations of adult parasites.

(ii) Serum Protein Determinations

Total serum protein was estimated by a biuret method (Weichselbaum 1946), on samples collected 24 hours prior to injection of the labelled albumin. Individual fractions were determined by electrophoresis on cellulose acetate following staining with Ponceau S and scanning with a Chromoscan.

(iii) Preparation and Labelling of Albumin

Labelling with ^{131}I was carried out by the method of McFarlane (1958) and immediately after labelling, carrier albumin was added to bring the specific activity of the labelled protein to below 5 μc/mg. The labelled preparation was then dialysed for 48 hours against 40 litres of saline and finally centrifuged prior to injection.

(iv) Drinking Water

To ensure rapid appearance in the urine of ^{131}I from degraded protein, the drinking water of each animal was replaced by a solution containing 0.005% NaI and 0.47% NaCl. This solution was given throughout

the experiment starting 3 days before injecting the labelled protein.

(v) Injection of Labelled Albumin and Blood Sampling

A volume of 5 ml. of labelled albumin solution containing about 350 μc ^{131}I was injected into the marginal ear vein of each of the experimental rabbits. Blood samples were collected from the opposite ear into tubes containing heparin as anticoagulant. The first sample was withdrawn 5 minutes after injection, a further 8 samples collected at suitable intervals over the first 4 days, and then daily for a further period of 7 days. Standard solutions were prepared by dilution of an aliquot of the labelled albumin solution used for injection.

(vi) Collection of Urine and Faeces

Urine and faeces were collected at regular 24-hour intervals following injection of the labelled albumin, the total output of each measured, and suitable samples taken for radioactivity determinations.

(vii) Radioactivity Measurements

Count rates of samples of urine, faeces and plasma were determined in a well-type scintillation counter. The volumes of plasma used varied from 0.25 to 0.5 ml. according to the stage of the experiment, but were always diluted to 5 ml. for counting. Suitable aliquots of the standard solutions, again in 5 ml. volumes, were assayed at regular intervals in order to correct count rates obtained for radioactive decay.

(viii) Construction of Curves

(a) Plasma Activity (Q_P)

The count rate per ml. of each plasma sample, corrected for radioactive decay was expressed as a percentage of the 5 minute post-injection sample and a semi-logarithmic plot made of activity against time. Curves obtained for one of the normal and one of the fluke-infected rabbits are shown (Fig. 6 and 7 respectively).

(b) Retained Activity (Q_R)

The activity retained in the body at the end of each 24-hour period following injection was obtained by subtracting the cumulative activity excreted in both urine and faeces from the injected dose. Curves were drawn byplotting daily retained activities as a percentage of the injected activity on a semi-logarithmic scale (Figs. 6 and 7).

(c) Extravascular Activity (Q_E)

The activity present in the extravascular compartments cannot be determined directly, but an indirect estimate can be made from Q_R and Q_P since

$$Q_R = Q_P + Q_E$$

Q_E was calculated as the difference between Q_R and Q_P at the end of each collection period (Figs. 6 and 7).

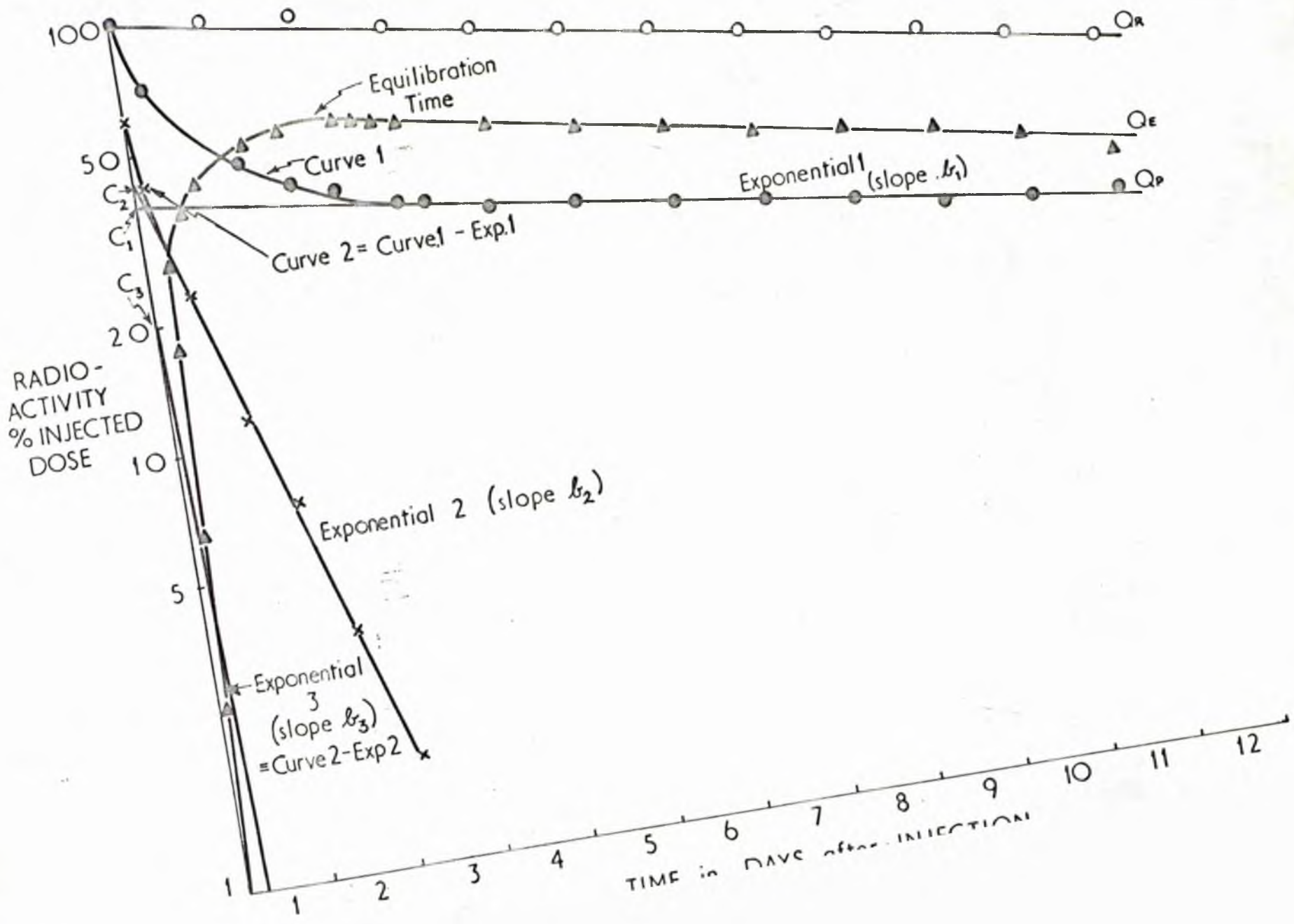
(viv) Injection of Labelled Polyvinylpyrrolidone and Blood Sampling

Five infected and five control rabbits were each injected with 1 ml. (175 μc) of ^{131}I-labelled polyvinylpyrrolidone (P.V.P.) obtained from the Radiochemical Centre, Amersham. Heparinised blood samples were

TURNOVER of ^{131}I-LABELLED ALBUMIN in NORMAL and FLUKE INFECTED RABBITS

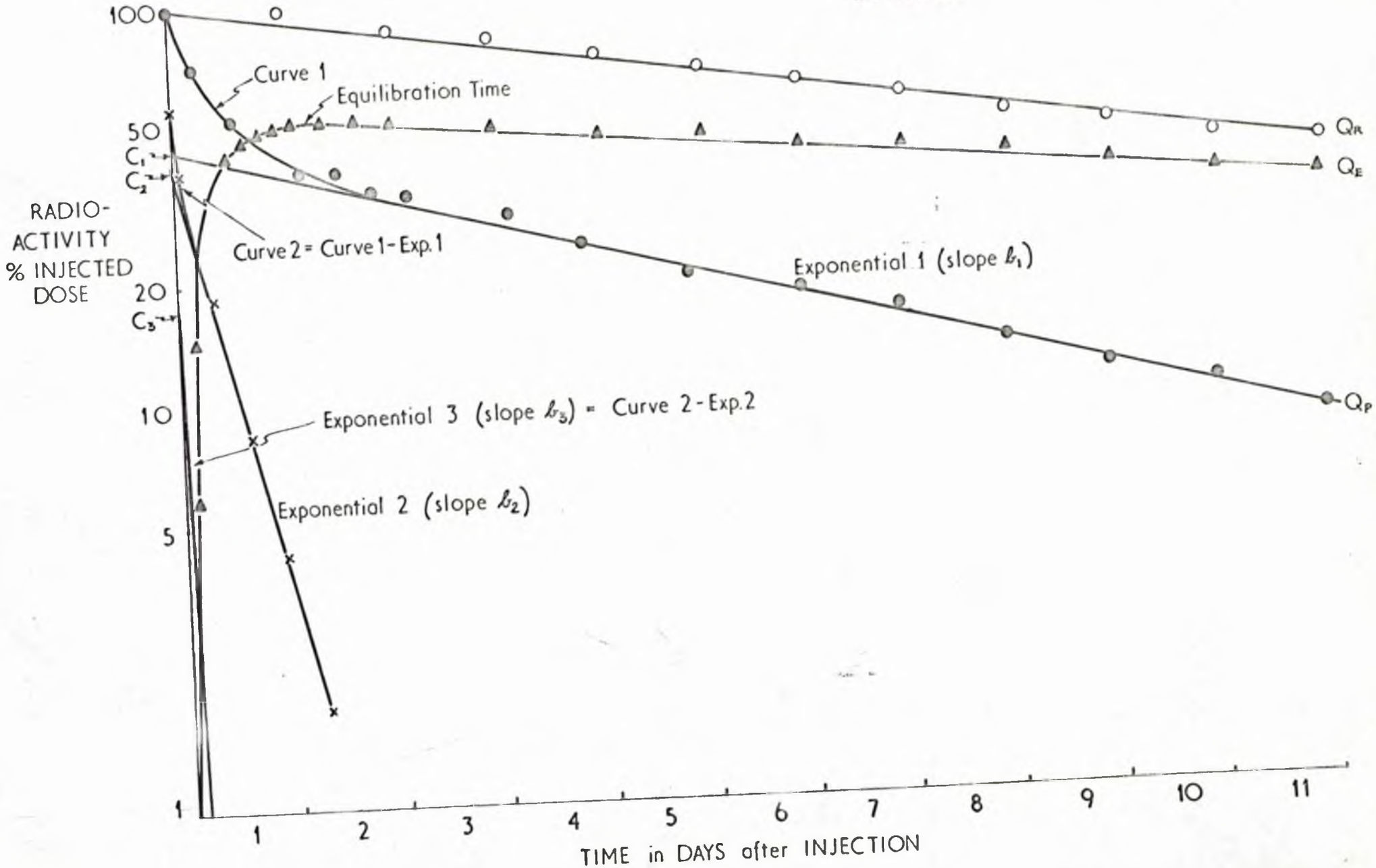
RABBIT No. 081 NORMAL

FIGURE 6



TURNOVER of ^{131}I-LABELLED ALBUMIN in NORMAL and FLUKE-INFECTED RABBITS
RABBIT No. 083 - INFECTED

FIGURE 7



collected twice daily for 5 days and a standard prepared from the original P.V.P. solution.

RESULTS

(A) BLOOD COMPOSITION OF NORMAL AND INFECTED RABBITS

A marked reduction in P.C.V. and serum albumin concentration was observed in the infected relative to the control group of animals (Table 1). It is noteworthy that there was no apparent decrease in total serum protein levels due to an elevated serum globulin concentration, this being mainly associated with the gammaglobulin fraction. Thus from a purely superficial examination of the blood of these animals, it can be concluded that anaemia, hypoalbuminaemia, and perhaps hyperglobulinaemia were the most apparent signs of infection.

These changes in blood protein chemistry yield no information on the total amount of for example, albumin in the body or of its distribution throughout the various compartments. This is obviously very necessary in order to eliminate such possibilities as an increased plasma volume or abnormal distribution of the protein as being the primary cause of the reduced serum concentrations observed, and can only be obtained by following the fate of intravenously injected labelled protein.

(B) ALBUMIN DISTRIBUTION IN NORMAL AND FLUKE-INFECTED RABBITS

(i) Plasma Volume (V_p)

The plasma volume was determined at the beginning of the study by the isotope "dilution principle". After injection of the labelled albumin, 5 minutes were allowed for complete mixing within the circulation and a blood sample withdrawn. The radioactivity per ml. of plasma was then divided into the injected radioactivity to obtain the plasma volume. In

Albumin Turnover Studies in Normal and Fluke-Infected Rabbits.

TABLE 1

PACKED CELL VOLUMES AND SERUM PROTEIN DATA

	Rabbit No.	P.C.V.	Total Protein (gm%).	Albumin (gm%).	Globulin (gm%)	A/G	Globulin (gm%)		
							Alpha	Beta	Gamma
CONTROL	082	37	6.00	3.45	2.55	1.33	0.38	1.10	1.07
	081	42	5.04	2.68	2.36	1.14	0.20	1.13	1.03
	890	37	6.75	3.50	3.25	1.08	0.45	1.77	1.03
	990	41	6.98	3.93	3.05	1.29	0.42	1.43	1.20
	Mean	39.	6.19	3.39	2.80	1.21	0.36	1.36	1.08
	S.D.	2.6	0.87	0.52	0.42	0.12	0.11	0.31	0.08
INFECTED	083	32	5.94	2.70	3.24	0.83	0.46	1.63	1.15
	334	28	6.62	2.66	3.96	0.67	0.55	1.39	2.02
	978	21	5.15	2.64	2.51	1.07	0.38	1.20	0.93
	179	26	5.15	2.18	2.97	0.73	0.46	1.34	1.37
	Mean	27	5.27	2.68	3.17	0.83	0.46	1.37	1.37
	S.D.	4.6	0.71	0.50	0.61	0.18	0.07	0.22	0.47
	P.	<0.01	N.S.	<0.05	N.S.	<0.02	N.S.	N.S.	N.S.

comparing the plasma volumes of the two groups of animals, both absolute values and those expressed on a body weight basis were used. The results of plasma volume estimates are shown (Table 2). It is apparent that no significant difference existed between the groups.

(ii) Intravascular Albumin (CA)

The amount of intravascular albumin was calculated from the plasma volume and the serum albumin concentration of a blood sample removed immediately prior to injecting the isotope.

$CA\ (gm.) = V_p \times \text{Serum Albumin}(\text{gm/ml})$.

Absolute values expressed on a body weight basis were used to compare albumin masses of the body compartments.

(iii) Total Body Albumin (TA)

Two methods (the "extrapolation" method and "equilibrium time" method) were used to determine the total body mass of albumin. In each case the size of the extravascular pool (EA) was calculated as the difference between TA and CA (TA = CA + EA). The extravascular/intravascular and extravascular/total albumin mass distribution ratios were independently calculated using the data obtained by both methods.

(a) Extrapolation Method (Figs. 6 and 7)

Following injection of ^{131}I-labelled albumin, the plasma concentration of the label (Q_p) initially fell rapidly due mainly to its distribution throughout the albumin pools of the body. This was followed by a phase during which the plasma activity declined as an exponential function of time. Assuming that no further redistribution of the label occurred during this

Albumin Turnover Studies in Normal and Fluke-Infected Rabbits

TABLE 2

PLASMA VOLUMES

	RABBIT No.	PLASMA VOLUME ml.	ml/kg.
CONTROL	082	152	43.4
	081	125	34.2
	890	84.1	36.6
	990	107.6	35.3
	Mean	117.2	37.4
	S.D.	28.6	4.1
INFECTED	083	108.7	35.1
	334	115.9	39.0
	978	122.2	39.4
	179	103.3	39.0
	Mean	111.0	38.1
	S.D.	5.4	2.0
	P	N.S.	N.S.

time, the intercept C_1, obtained by extrapolating the linear part of the curve to the ordinate, indicated that fraction of the total albumin which would have been present in the plasma at zero time if equilibration of the label between the compartments had occurred instantaneously (Sterling 1951).

$$\text{i.e. TA (gm/Kg)} = \frac{\text{CA(gm/kg)}}{C_1}$$

The albumin pool masses and distribution ratios obtained by this method are shown (Table 3). A mean reduction of 25% in the total albumin pool was associated with infection. This was due mainly to the marked decrease in the extravascular mass compared to a small (and insignificant) drop in the intravascular pool. No significant difference was found in albumin distribution, although as a result of the small extravascular pools, the extravascular/intravascular mass distribution ratios of the infected group were an average 20% lower than those of the controls.

(b) Equilibrium Time Method

The extravascular activity curve (Q_E), obtained as the difference between Q_R and Q_p rose to a maximum and subsequently declined at a similar rate as Q_p (Figs. 6 and 7). When Q_E is maximal, the albumin specific activity in all compartments was equal and no net transfer of labelled molecules occurred across the capillary boundaries, i.e. the labelled albumin had completely equilibrated with the total exchangeable albumin pool. At this equilibrium time, the ratio between extravascular and intravascular activity equaled the ratio between the extravascular and intravascular pool masses, (Campbell et al 1956).

Albumin Turnover Studies in Normal and Fluke-Infected Rabbits

Distribution of Albumin between vascular and extravascular pools

TABLE 3

POOL SIZES AND DISTRIBUTION RATIOS (STERLING)

	RABBIT NO.	CA (Gm./Kg.)	EA (Gm./Kg)	TA (Gm./Kg)	EA/CA	EA/TA
CONTROL	082	1.49	1.61	3.10	1.08	0.52
	081	0.90	1.41	2.30	1.57	0.61
	890	1.27	2.01	3.28	1.58	0.61
	990	1.38	2.18	3.56	1.58	0.61
	Mean	1.26	1.80	3.06	1.45	0.59
	S.D.	0.26	0.35	0.54	0.25	0.05
INFECTED	083	0.94	1.14	2.08	1.21	0.55
	334	1.15	1.60	2.75	1.38	0.58
	978	1.03	1.25	2.28	1.21	0.55
	179	0.84	1.01	1.85	1.20	0.55
	Mean	0.99	1.25	2.24	1.25	0.56
	S.D.	0.14	0.25	0.39	0.09	0.01
	P	N.S.	<0.05	<0.05	N.S.	N.S.

i.e. $\frac{Q_E}{Q_p} = \frac{EA}{CA}$

and since $TA = CA + EA$

$$TA = \frac{CA\ (Q_p + Q_E)}{Q_p}$$

Values for TA (and hence EA) calculated by this procedure were almost identical to those obtained by the extrapolation method, but on average were 10% higher in the infected group (Table 4).

Conclusions

The theoretical assumptions upon which calculation of distribution ratios by the extrapolation and equilibrium time methods are based differ significantly. The extrapolation procedure assumes that protein synthesis and catabolism take place in both the intra- and extravascular compartments, that no significant redistribution of labelled protein occurs after initial equilibration is complete and that specific activities (i.e. counts/mg. protein) are equal throughout the total body pool of the protein under study. This is not the case. It is apparent (Figs. 6 and 7) that as injected labelled molecules first enter the various interstitial fluids, the mean extravascular protein specific activity increases while its value in the plasma falls. Only when the extravascular activity is maximal can specific activities in all compartments be assumed to be equal. Thereafter they could only remain equal if metabolic breakdown occurred in all compartments in proportion to the mass of protein in each. It is now known that this is not the case and that catabolism from a functional point of view is "intravascular". Thus after the preliminary distribution period, the specific activity of the plasma compartment will always be lower than that

Albumin Turnover Studies in Normal and Fluke-infected Rabbits

Distribution of Albumin between Vascular and Extravascular Pools

TABLE 4

POOL SIZES AND DISTRIBUTION RATIOS (CAMPBELL)

	RABBIT NO.	EQUILIBRIUM TIME(HOURS)	CA (gm/kg)	EA (gm/kg)	TA (gm/kg)	EA/CA	EA/TA
CONTROL	082	66	1.49	1.64	3.13	1.10	0.52
	081	52	0.90	1.33	2.23	1.48	0.60
	890	48	1.27	2.01	3.28	1.58	0.61
	990	38	1.38	1.61	2.99	1.17	0.54
	Mean	51	1.26	1.65	2.91	1.33	0.57
	S.D.	11.6	0.26	0.28	0.47	0.24	0.04
	$P_{S/C}$	-	-	N.S	N.S	N.S	N.S.
INFECTED	083	30	0.94	1.28	2.22	1.36	0.58
	334	30	1.16	1.79	2.95	1.54	0.61
	988	38	1.03	1.67	2.70	1.62	0.62
	179	34	0.84	1.32	2.16	1.57	0.61
	Mean	33	0.99	1.52	2.51	1.52	0.61
	S.D.	3.6	0.14	0.25	0.38	0.11	0.02
	$P_{S/C}$	-	-	N.S	N.S	N.S	N.S.
	P	< 0.05	N.S.	N.S.	N.S.	N.S.	N.S.

$P_{S/C}$ - Significance of difference between Sterling and Campbell methods of analysis.

in the extravascular compartments, resulting in a net transfer of protein of higher specific activity from the extravascular pools back into the plasma. This transfer will have the effect of decreasing the slope of the plasma activity curve. Extrapolation of the linear part of the curve to the ordinate will yield values for C_1, which are erroneously low and therefore values obtained for TA and EA will be overestimated.

The magnitude of the error introduced by using Sterling's procedure will depend on the differences in specific activity between compartments. Although the amount of radioactivity present extravascularly in the normal animals (Fig. 6), was about 10% greater than that in the intravascular compartment, the parallel decline in the activity associated with these compartments, together with the larger extravascular mass of protein, indicated that in these animals specific activity differences were not great. Thus no serious analytical error will result if specific activities are regarded as being uniform throughout the exchangeable albumin pool after the initial period of equilibration.

In the case of the infected animals however this assumption would appear to be more dubious because of the marked differences in activity associated with the intra- and extravascular pools (Fig. 7). The significance of this difference is difficult to assess since retention of radioactive breakdown products is known to occur following catabolism of radioiodinated proteins which have a short biological half-life e.g. fibrinogen (McFarlane 1963 (b)). In his studies values obtained for non-precipitable portions of the plasma activities indicated that 24 - 32% of

the total retained activity was present as iodide in the body water. Thus although proportions of non-precipitable activity in the plasma seldom rise to significant levels, because the body iodide pool is about 8 times larger than the plasma pool the effect of retained iodide on the total body activity is much greater. This is the probable explanation for the observed slower decline of Q_R relative to Q_P, and since Q_E is determined by "difference" from Q_R and Q_P this accounts for the divergence of Q_R and Q_E.

The equilibrium time method is based on the assumptions that the protein is catabolised intravascularly, and that the radioactive iodine after liberation from the degraded protein is immediately excreted. Although the method is relatively simple, it can be troublesome in that it requires accurate estimation of the equilibrium time from the extravascular activity curve where the curvature is so gradual that it is difficult to accurately select the point of maximal radioactivity. It is clear that significant retention of iodide will lead to a marked overestimation of Q_E and therefore of TA. Thus although the values calculated by the two methods were very similar, the slight increase in TA values calculated by the equilibrium time method over those determined by the extrapolation procedure was probably associated with iodide retention.

The significant reduction in the time taken for the labelled albumin to equilibrate with the total body pool of protein in the infected group was consistent with their reduced albumin pool masses. The fact that the two methods used in the present study give very similar results suggests that

the assumptions upon which they are based are justifiable for the calculation of distribution ratios.

Having established that the reduced serum albumin levels associated with the disease were due to an over-all reduction in albumin pool mass and not to extravascular distribution of the protein, it was then necessary to calculate the turnover of this protein in order to assess the relative importance of subnormal synthesis or increased catabolism as the primary influence in causing the hypoalbuminaemia.

(C) CATABOLISM OF ^{131}I-LABELLED ALBUMIN IN NORMAL AND FLUKE-INFECTED RABBITS

The catabolic rates of plasma proteins can be estimated by applying different mathematical approaches to the analysis of the data. These "models" require assumptions of variable validity. Generally two broad types of model are employed - those which assume that synthesis and catabolism occur in both intra- and extravascular compartments and those which are based on the assumption of intravascular degradation. Using either or both model types, catabolism can be calculated "indirectly" by analysis of the plasma activity disappearance curve or "directly" from excreted radioactivity.

Although large day to day variations in results based on excreted activity may occur it should be borne in mind that these do not represent variations in catabolic rate, but rather variations in voiding of urine containing the isotope released by breakdown of the label.

(i) Models which Assume Intravascular and Extravascular Degradation of Plasma Proteins

(a) Analysis of Plasma Activity Curve (Sterling 1951)

Following injection of ^{131}I-labelled albumin, plasma activity follows a definite pattern with time (Figs. 6 and 7). Initially, the concentration of the label falls rapidly, and this is followed 2 - 3 days later by a phase in which decline is logarithmic with time. The steep part of the curve is mainly due to the labelled albumin leaving the circulation and becoming distributed extravascularly. In addition however since some of the labelled protein will have been catabolised during this period, the rapid decline also reflects protein degradation. The plasma activity then falls exponentially and Sterling (1951) considered that this phase represented solely catabolism of the labelled protein and that its slope could be used to calculate the "half-life" ($T_{\frac{1}{2}}$) of the protein, i.e. the time taken for the plasma activity to fall by 50%.

It is apparent from the plasma activity curves that the rate of disappearance of the label from the circulation of the infected and control animals were markedly different, this taking the form of a greatly reduced "half-life" value in the infected over the control group (Table 5).

Sterling (1951) further considered that the turnover rate k of the protein concerned could be calculated from the "half-life" and slope of the final exponential.

$$\text{i.e. } k = \frac{0.693}{T_{\frac{1}{2}}(\text{days})} \text{ days}^{-1}$$

Since the exponential part of the plasma activity curve does not begin until the labelled albumin is evenly distributed throughout all the body albumin pools, k indicates that fraction of the total body pool catabolised per day F(TA).

Albumin Turnover in Normal and Fluke-Infected Rabbits

TABLE 5

APPARENT HALF-LIFE OF LABELLED ALBUMIN AND CATABOLIC RATE
(STERLING)

	RABBIT NO.	$T_{\frac{1}{2}}$(hrs)	k($days^{-1}$)	Absolute Amount Catabolised (gm/kg/day)
CONTROL	082	260	0.064	0.198
	081	233	0.071	0.163
	890	185	0.090	0.295
	990	165	0.101	0.356
	Mean	211	0.082	0.253
	S.D.	44	0.014	0.089
INFECTED	083	107	0.155	0.322
	334	80	0.208	0.574
	978	76	0.219	0.502
	179	60	0.277	0.512
	Mean	81	0.215	0.478
	S.D.	20	0.049	0.108
	P	< 0.002	< 0.002	< 0.02

Since both the fractional catabolic rate and pool mass of the total body albumin are now defined, the mass of albumin catabolised per day can be calculated.

Mass of albumin catabolised (gm/kg/day) = TA x F(TA)

Values obtained for F(TA) and the absolute amounts of albumin catabolised show that a marked difference existed between the groups in the degradation rate which on average is double in the infected rabbits (Table 5).

(b) Analysis of Excreted Radioactivity (Berson et al 1953)

The fractional catabolic rate of labelled albumin (as a percentage of the total pool) can alternatively be determined by expressing the daily excreted radioactivity as a fraction of the average amount retained in the same 24-hour period.

$$F(TA) = \frac{\text{Total Excreted Activity}}{\text{Mean Retained Activity}}$$

Excretion of radioactivity during the first day of the study was not used for the calculation because of the influence of the iodide pool, but the results obtained for each day thereafter are shown (Table 6).

The difference between the groups was barely significant ($P = 0.05$) when calculated by this method compared to the marked hypercatabolism in the infected rabbits when determined from the plasma activity curve.

The accuracy of estimations of degradation from excreted activity are dependent on the important assumption that the label is excreted immediately after liberation from the protein. This assumption was not valid at least in the case of the infected animals because the fall in Q_R

Turnover of ^{131}I-labelled Albumin in Normal and Fluke-infected Rabbits

TABLE 6

F(TA) - FRACTION OF TOTAL BODY POOL CATABOLISED/DAY

Day	082	890	081	990	083	179	978	334
2	0.066	0.065	0.087	0.164	0.130	0.174	0.152	0.091
3	0.092	0.090	0.166	0.109	0.092	0.163	0.084	0.145
4	0.003	0.066	0.077	0.042	0.109	0.146	0.168	0.280
5	0.082	0.071	0.074	0.106	0.099	0.124	0.109	0.190
6	0.002	0.077	0.078	0.086	0.092	0.103	0.097	0.092
7	0.016	0.050	0.084	0.122	0.109	0.111	0.100	0.115
8	0.037	0.062	0.082	0.101	0.102	0.075	0.073	0.100
9	0.004	0.044	0.069	0.057	0.078	0.007	0.068	0.072
10	0.123	0.050	0.105	0.120	0.073	0.132	0.061	0.114
11	0.001	0.052	0.063	0.116	0.074	0.066	0.032	0.095
Mean	0.043	0.062	0.088	0.102	0.096	0.110	0.095	0.129
S.D.	0.044	0.010	0.028	0.033	0.014	0.049	0.040	0.062

did not parallel that of Q_p, indicating that retention of iodide in the body water occurred. The extent of iodide retention was estimated by comparing the slope constants of Q_R and Q_p (Table 7). Almost identical values were obtained in normal rabbits indicating that F(TA) could be calculated from either Q_p, Q_R or analysis of excreted radioactivity.

This was not the case for the infected animals since although values obtained for catabolism based on the slope of Q_R or directly from excreted activity gave identical results, those estimated from the slope of the plasma disappearance curve were significantly greater because of the divergence between Q_p and Q_R. It is clear that marked iodide retention, because of its effect of both reducing excretion and increasing retention of isotope was responsible for the reduced values obtained for F(TA) in the infected animals. It is doubtful if the iodide retention was due to incomplete collection of urine and faeces since the same procedure was adopted for each animal. The only other possible explanation is thyroid uptake although it is difficult to explain why this only occurred in the infected group since all the rabbits appeared to drink similar amounts of inactive iodide-containing water. McFarlane (1963(b)) noted a similar retention in rabbits following injection of ^{131}I-labelled fibrinogen (apparent half-life 0 hours). It would therefore appear that this phenomenon is peculiar to protein with a rapid turnover rate, although the reason is unknown.

(ii) Models which Assume Intravascular Degradation

Since it has been shown that albumin is catabolised in or near the

TABLE 7

F(TA) - FRACTION OF TOTAL BODY POOL DEGRADED / DAY

	RABBIT NO.	CALCULATED FROM		
		DAILY EXCRETED ACTIVITY	Q_R	Q_P
CONTROL	082	0.043	0.054	0.064
	081	0.088	0.086	0.071
	890	0.062	0.065	0.090
	990	0.102	0.128	0.101
	Mean	0.074	0.083	0.082
	S.D.	0.024	0.032	0.014
INFECTED	083	0.096	0.088	0.155
	334	0.129	0.130	0.208
	978	0.095	0.098	0.219
	179	0.110	0.124	0.277
	Mean	0.108	0.110	0.215
	S.D.	0.014	0.017	0.049
	P	0.05	N.S.	<0.002

plasma, it seems more pertinent to express the turnover rate as a fraction of the intravascular pool. Moreover, because the intravascular protein mass can be determined much more accurately than the total, calculation of the absolute amount of protein catabolised is much less liable to serious error.

Two methods are commonly used to measure catabolism - the "indirect" method of Matthews (1957), which is based entirely on the plasma disappearance curve, and the method first suggested by Campbell et al (1956), which relates degradation to excreted radioactivity.

It was previously pointed out that the main drawback of the Sterling method is that it assumes that the plasma activity curve reflects only catabolism of the labelled protein. In fact redistribution of protein between the intra- and extravascular compartments also occurs and this has the effect of increasing the apparent half-life of the protein, thereby yielding an erroneously small fractional catabolic rate. Determination of the true value depends on a knowledge of the rate of redistribution of the labelled molecules between the compartments. This information can be obtained from graphic analysis of the plasma activity curve.

(a) Graphic Analysis of the Plasma Activity Curve

This method of calculating protein catabolism is based only on the plasma disappearance curve of the labelled protein plotted on a semilogarithmic scale. The method assumes that the body albumin is distributed throughout an open mammillary compartment system consisting of a central compartment (the plasma) which is reversibly connected with several outer compartments

(representing the extravascular pools). It is assumed that protein degradation occurs only in the intravascular compartment and that the total mass of protein in each compartment and the rates of transfer from one compartment to another are constant, i.e. that "steady-state" conditions are operating.

It was previously shown that after equilibration with the extravascular pools, the plasma activity curve becomes a straight line, so that intravascular activity was a single exponential function of time. This function is assumed to be $C_1 \exp(-b_1 t)$ where b_1 is the slope of the line (i.e. $\frac{0.693}{T_{\frac{1}{2}}(\text{days})}$) and C_1 its intercept with the ordinate, obtained by extrapolating the linear phase to t_o (Figs. 6 and 7). Subtraction of this extrapolated line from the original curve at suitable time intervals gives a second curve which also becomes linear after some time. This line or curve represents transfer of labelled albumin to the extravascular compartments, and the linear part can similarly be represented by the exponential function $C_2 \exp(-b_2 t)$, so that the slope of this line is $-b_2$ and C_2 is the point at which it cuts the ordinate. It was found that in most rabbits, a further exponential was required to fully describe the plasma activity curve, so that it was represented by an equation of the type.

$$\text{Plasma Activity} = C_1 \exp(-b_1 t) + C_2 \exp(-b_2 t) + C_3 \exp(-b_3 t)$$

Matthews (1957) has shown mathematically that the catabolic rate K, as a fraction of the intravascular pool broken down per day can be calculated from the equation:

$$K = \frac{1}{\frac{C_1}{b_1} + \frac{C_2}{b_2} + \frac{C_3}{b_3} + \ldots\ldots \frac{C_n}{b_n}}$$

The slope b_1 and intercept C_1 of the final exponential can usually be determined accurately, but b_2 and C_2 are more subject to error since they are essentially "difference" measurements. The constants of the third exponential are even more erroneous, and since they are only required to define the plasma activity curve over the first few hours following injection, they were excluded from any calculations of K. Since b_3 is much greater than b_2, the exclusion of the third exponential from the above equation will not significantly alter values obtained for K, and the following shortened formula could therefore be used for estimating catabolism by this method:

$$K = \frac{1}{\frac{C_1}{b_1} + \frac{C_2}{b_2}}$$

Values obtained for these constants were used as describes to calculate K. It is clear from the results in Table [illegible], that the catabolism of the labelled albumin, expressed as a fraction of the intravascular pool is dramatically greater in the fluke-infected rabbits. The values obtained for b_1 confirm that the finding of a reduced "apparent half life" is good qualitative (and when applied to this method of estimating catabolism) good quantitative evidence of increased degradation, since it is this factor above all others which determines the value obtained for K.

Turnover of Labelled Albumin in Normal and Infected Rabbits

TABLE 8

K - CATABOLIC RATE (MATTHEWS)

	RABBIT No.	b_1	b_2	C_1	C_2	K
CONTROL	082	0.064	1.11	0.48	0.52	0.125
	081	0.071	1.38	0.38	0.42	0.177
	890	0.090	1.66	0.39	0.46	0.210
	990	0.101	1.51	0.39	0.42	0.243
	Mean	0.082	1.42	0.41	0.46	0.191
	S.D.	0.014	0.23	0.05	0.05	0.051
INFECTED	083	0.155	2.38	0.45	0.40	0.326
	334	0.208	3.32	0.42	0.43	0.465
	978	0.219	2.77	0.45	0.39	0.457
	179	0.277	2.68	0.48	0.52	0.485
	Mean	0.215	2.51	0.45	0.44	0.434
	S.D.	0.049	0.73	0.02	0.06	0.072
	P.	<0.002	<0.05	N.S.	N.S.	<0.002

It is also interesting to note that the values of b_2, which reflect the rate of disappearance of the label from the circulation over the first 2 - 3 days following injection are also markedly increased in the infected group. Although this is mainly due to increased catabolism of the labelled protein over this period, it also explains the previously reported finding of a reduced equilibrium-time in these animals.

(b) Analysis of Excreted Radioactivity

A more "direct" confirmatory method of analysis can be applied to the system based on activity excreted in the urine and faeces. This method, first introduced by Campbell et al (1956) assumes that the excreted output of isotope which arises from degradation of labelled protein will be proportional to the amount of ^{131}I-labelled albumin present in the pool where degradation takes place. Thus if it is assumed that protein catabolism occurs intravascularly the fraction of the plasma pool broken down each dayF(CA) can be determined from the daily excreted activity and the average activity present in the plasma within the same time.

$$F(CA) = \frac{\text{Total Excreted Activity}}{\text{Mean Plasma Activity}}$$

These values can then be used to calculate the mass of albumin catabolised per day within the vascular compartment.

Mass of Albumin Catabolised (gm/kg/day) = CA x F(CA)

Albumin catabolic rates expressed either as percentages of the intravascular pool or as absolute amounts were clearly very much higher in the infected rabbits ($P < 0.02$.) (Tables 9 and 10).

Turnover of ^{131}I - Labelled Albumin in Normal and Fluke-Infected Rabbits

TABLE 9

F(CA) - CATABOLIC RATE (CAMPBELL)

	Control				Infected			
Day	082	390	081	990	083	179	978	334
2	0.113	0.157	0.192	0.354	0.318	0.472	0.398	0.245
3	0.188	0.243	0.425	0.254	0.246	0.503	0.258	0.411
4	0.006	0.191	0.194	0.097	0.309	0.506	0.559	0.882
5	0.173	0.201	0.184	0.234	0.302	0.487	0.435	0.654
6	0.004	0.217	0.192	0.168	0.308	0.465	0.437	0.341
7	0.034	0.152	0.206	0.251	0.378	0.569	0.504	0.450
8	0.079	0.187	0.204	0.201	0.395	0.445	0.416	0.422
9	0.001	0.138	0.162	0.111	0.311	0.049	0.442	0.341
10	1.111	0.165	0.260	0.216	0.305	1.008	0.446	0.559
11	0.003	0.173	0.151	0.206	0.329	0.577	0.272	0.524
Mean	0.171	0.182	0.217	0.209	0.320	0.509	0.416	0.483
S.D.	0.338	0.030	0.078	0.073	0.040	0.230	0.092	0.183

$P < 0.01$

Turnover of ^{131}I-labelled Albumin in Normal and Fluke-infected Rabbits

TABLE 10

ABSOLUTE AMOUNTS OF ALBUMIN CATABOLISED (GM./KGM./DAY)

	Control				Infected			
Day	082	890	081	990	083	179	978	334
2	0.168	0.199	0.172	0.488	0.298	0.396	0.410	0.284
3	0.280	0.308	0.382	0.350	0.232	0.422	0.266	0.477
4	0.010	0.242	0.175	0.132	0.291	0.428	0.576	1.023
5	0.257	0.255	0.166	0.322	0.284	0.418	0.448	0.758
6	0.006	0.275	0.173	0.231	0.289	0.390	0.447	0.396
7	0.050	0.192	0.185	0.346	0.355	0.477	0.516	0.522
8	0.118	0.237	0.184	0.278	0.371	0.374	0.428	0.489
9	0.001	0.176	0.147	0.153	0.292	0.041	0.456	0.396
10	1.655	0.209	0.233	0.297	0.286	0.846	0.459	0.648
11	0.004	0.219	0.136	0.289	0.309	0.468	0.260	0.608
Mean	0.255	0.231	0.195	0.288	0.201	0.426	0.429	0.562
S.D.	0.477	0.037	0.066	0.102	0.035	0.182	0.089	0.201

p 7 0.005

Conclusions

In normal rabbits specific activity differences between intra- and extravascular pools are not large and therefore estimates of catabolic rates based on plasma activity curves are an accurate assessment of degradation. Similarly, those calculated from excreted activity are equally valid because of rapid and quantitative excretion of isotope following breakdown of the label.

It is clear that in these animals the assumption of intravascular degradation is valid since catabolic rates of the labelled albumin expressed either as a fraction of the total body or intravascular pool were almost identical when calculated as absolute amounts (0.253 and 0.245 gm/kg/day respectively).

This insignificant difference is due to the iodide pool which slightly delays excretion of the label following catabolism of iodine-labelled proteins, and it is for this reason that some authors have incorporated a "breakdown products" pool (representing the body iodide pool) into which the radioiodine is discharged prior to excretion (Reeve and Roberts 1959; Nosslin 1962). Since the excretion rate of iodide in the rabbit normally varies between 1.5 - 3.5 days^{-1} (Zizza et al 1959) the error involved in excluding an iodide pool from the mathematical model is very small. Provided the slope constants of Q_P and Q_R are approximately equal, the error on the fractional catabolic rate is given by b_1/k_5 where k_5 is the iodide excretion rate (Andersen 1964).

In each of the normal rabbits this error was not greater than 5% but it does explain the slightly increased fractional catabolic rates normally obtained by analysis of excreted activity. It is therefore apparent that accurate estimates of albumin catabolism can be obtained for normal animals from analysis of plasma activity curves, thereby rendering these methods particularly useful for experiments on sheep and cattle where quantitative collection of urine and faeces is often difficult.

In the infected rabbits however catabolic rates claculated from either the slope of the plasma activity disappearance curve or by analysis of excreted activity are underestimated. Specific activity differences between the plasma and extravascular compartments have the effect of prolonging the exponential part of the plasma activity curve beyond its true value, thereby reducing the values obtained for F(TA) by the Sterling method and F(CA) by graphic analysis below their true value. As a result of iodide retention, the amount of activity excreted each day did not truly reflect the quantity of albumin catabolised and thus values obtained for F(TA) and F(CA) by analysis of excreted activity also underestimated catabolism. The error involved in calculating F(CA) is not as great as that in calculating F(TA) by this method because the eight-fold difference in the size of the intravascular and total body iodide poolshas the effect of reducing the slope of C_R to a much greater extent than that of C_P.

Several interesting observations arose from the values calculated for F(CA) in infected animals. In the first place, very good agreement

was obtained between estimates of catabolism based on analysis of the plasma activity curve and those calculated from excreted activity. As in the case of normal rabbits the assumption of intravascular degradation is equally valid since the amount of albumin catabolised in the intravascular compartment (mean 0.429 gm/kg/day) could account for that degraded throughout the body (mean 0.478 gm/kg/day).

The most striking and consistent feature of these results however was the high catabolic rate of albumin in the infected rabbits, expressed either as a fraction of the total or intravascular pool.

(D) AETIOLOGY OF ALBUMIN HYPERCATABOLISM IN FLUKE-INFECTED RABBITS

While the above results show an abnormally high rate of breakdown of the labelled albumin in infected rabbits, they give no indication as to how this situation arises. The finding of Jennings et al (1956) of high radioactivity associated with flukes removed from rabbits previously injected with ^{131}I-labelled albumin indicated the possibility of protein loss into the gut via the bile as being the cause of the hypoalbuminaemia associated with the disease.

Because of this possibility the mean radioactivity of the faeces in both groups of animals was calculated. To correct for differences in plasma activity, this result was estimated in the form of a "clearance" i.e. it represents the volume of plasma which would have to appear in the faeces in the 24-hour collection period to account for the radioactivity. Plasma "clearances"were calculated by dividing the total radioactivity in each 24-hour collection of faeces by the activity per ml. of plasma taken

at the beginning of the collection period (Table 11). This ratio was clearly higher in the infected than in the control animals. (Values obtained for each day of the study are shown in Appendix A.)

Although these values provide good qualitative evidence of protein loss into the alimentary tract, they are quantitatively a gross underestimate because of the substantial breakdown and reabsorption of the label which is known to occur with radioiodinated plasma protein.

(E) USE OF ^{131}I-LABELLED POLYVINYLPYRROLIDONE (P.V.P.) FOR ESTIMATION OF MOVEMENT OF PLASMA MACROMOLECULES INTO THE GASTRO-INTESTINAL TRACT OF NORMAL AND FLUKE-INFECTED RABBITS

Direct measurement of the movement of plasma macromolecules into the gut is possible using ^{131}I-labelled polyvinylpyrrolidone (P.V.P. Fig. 4) since it is neither degraded by intestinal enzymes nor absorbed from the gut on oral administration. Five infected rabbits which had received 50 metacercariae 10 weeks previously and were showing signs of infection in the form of significant reductions in P.C.V. and serum albumin concentrations (Appendix A), and 5 control rabbits were injected with 175 μc of labelled P.V.P. and faeces and plasma collected and assayed for radioactivity. A semi-logarithmic plot of plasma activity against time was made. Curves obtained for one of the normal and one of the fluke-infected rabbits are shown in Fig. 8. The plasma half-life of the labelled P.V.P. was significantly shortened in infected rabbits (Table 12).

Turnover of ^{131}I - Labelled Albumin in Normal and Fluke-infected Rabbits

TABLE 11

Mean Daily Faecal Radioactivity Expressed as a "Plasma Clearance"

	Rabbit No.	Plasma Clearance (ml/24hrs)	S.D.
CONTROL	082	1.06	0.80
	081	0.51	0.14
	890	0.68	0.39
	990	1.68	0.85
	Mean	0.98	
	S.D.	0.52	
INFECTED	083	1.72	0.79
	334	3.68	2.79
	978	2.70	1.29
	179	2.77	1.08
	Mean	2.72	
	S.D.	0.80	
	P.	<0.02	

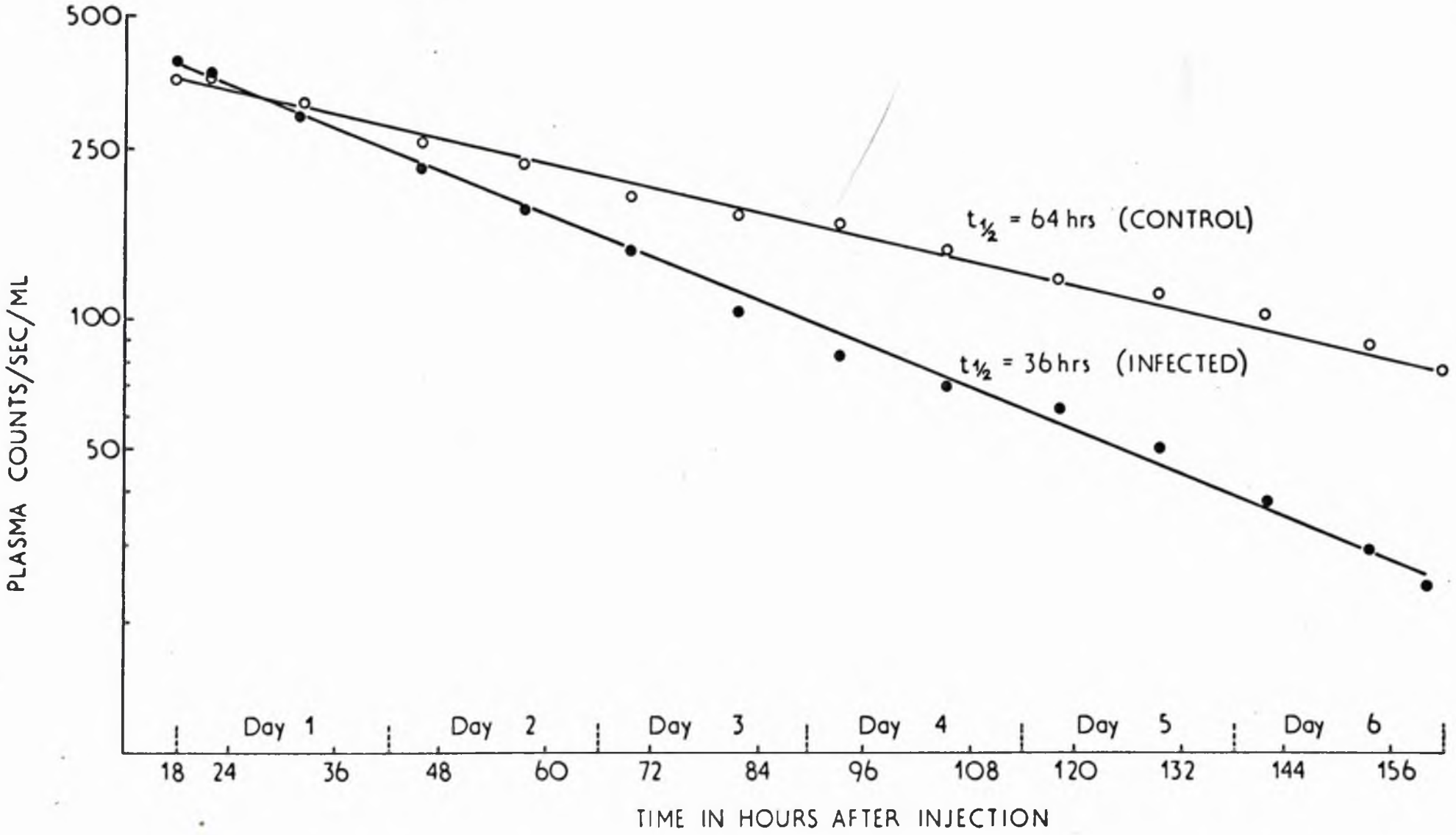
PLASMA "HALF-LIFE" AND FAECAL EXCRETION OF P.V.P. IN FLUKE-INFECTED AND NORMAL RABBITS

TABLE 12

	Rabbit No.	Half-life Plasma PVP (hr)	Cumulative (5 day) faecal output as % Injected dose.	Mean daily plasma "clearance"(faecal) (ml)
INFECTED	971	36	17.0	27.2
	084	50	10.3	19.7
	008	39	12.9	28.3
	996	43	16.9	32.8
	336	40	20.3	40.6
	Mean	42	15.5	29.7
	S.D.	5	3.9	7.7
CONTROL	985	88	2.1	2.3
	110	73	2.5	3.7
	199	65	4.0	4.7
	CLT	68	2.5	3.0
	203	60	4.1	5.1
	Mean	71	3.0	3.8
	S.D.	11	0.9	1.2
	P	< 0.001	< 0.001	< 0.001

FIGURE 8

^{131} I-PVP RABBIT EXPERIMENT



(i) Faecal Excretion of ^{131}I-labelled P.V.P. in Fluke-Infected and Normal Rabbits

Plasma "clearances" were calculated as previously described and the cumulative faecal activity excreted during the experiment expressed as a % of that injected. The results obtained (Table 12) show the marked difference between infected and control animals in the faecal output of P.V.P. expressed as cumulative faecal output or as a "clearance" figure, and indicate a considerable movement of plasma macromolecules into the alimentary tract. "Clearance" values obtained for each day of the study are shown in Appendix A.

(F) SIMULTANEOUS MEASUREMENT OF THE TURNOVER OF ^{125}I-LABELLED ALBUMIN AND ^{131}I-LABELLED 7S-GAMMAGLOBULIN IN NORMAL AND FLUKE-INFECTED RABBITS

MATERIALS AND METHODS

(i) Experimental Animals

Four rabbits each infected with 50 metacercariae 12 weeks previously and three normal rabbits were used in this study. The drinking water of all animals was replaced by a solution containing inactive carrier iodide (0.005% NaI and 0.47% NaCl), and urine and faeces were collected daily as for the previous experiment.

(ii) Preparation and Labelling of Protein

Labelling with ^{131}I and ^{125}I was carried out by the method of McFarlane (1958) and carrier albumin added to each labelled protein solution

to minimise radiation decomposition. Each preparation was dialysed against saline to remove unbound iodide and centrifuged prior to injection.

In order to check that each isotope was confined to the protein labelled 0.5 ml. of each of the labelled proteins was mixed with 1 ml. of normal rabbit serum and eluted through a column of Sephadex G-200 (35 cm x 3 cm). This separates the serum proteins into three distinct fractions on the basis of molecular weight. 5 ml. protions of the eluate were analysed for both protein and radioactivity, and the distribution of these confirmed that most of the ^{131}I activity was associated with the 7S-gammaglobulin, while the ^{125}I activity corresponded to the albumin fraction. (Fig. 9). The slight overlap in radioactivity was due to the unavoidable incomplete separation of 7S-gammaglobulin from albumin.

(iii) Injection of Labelled Proteins

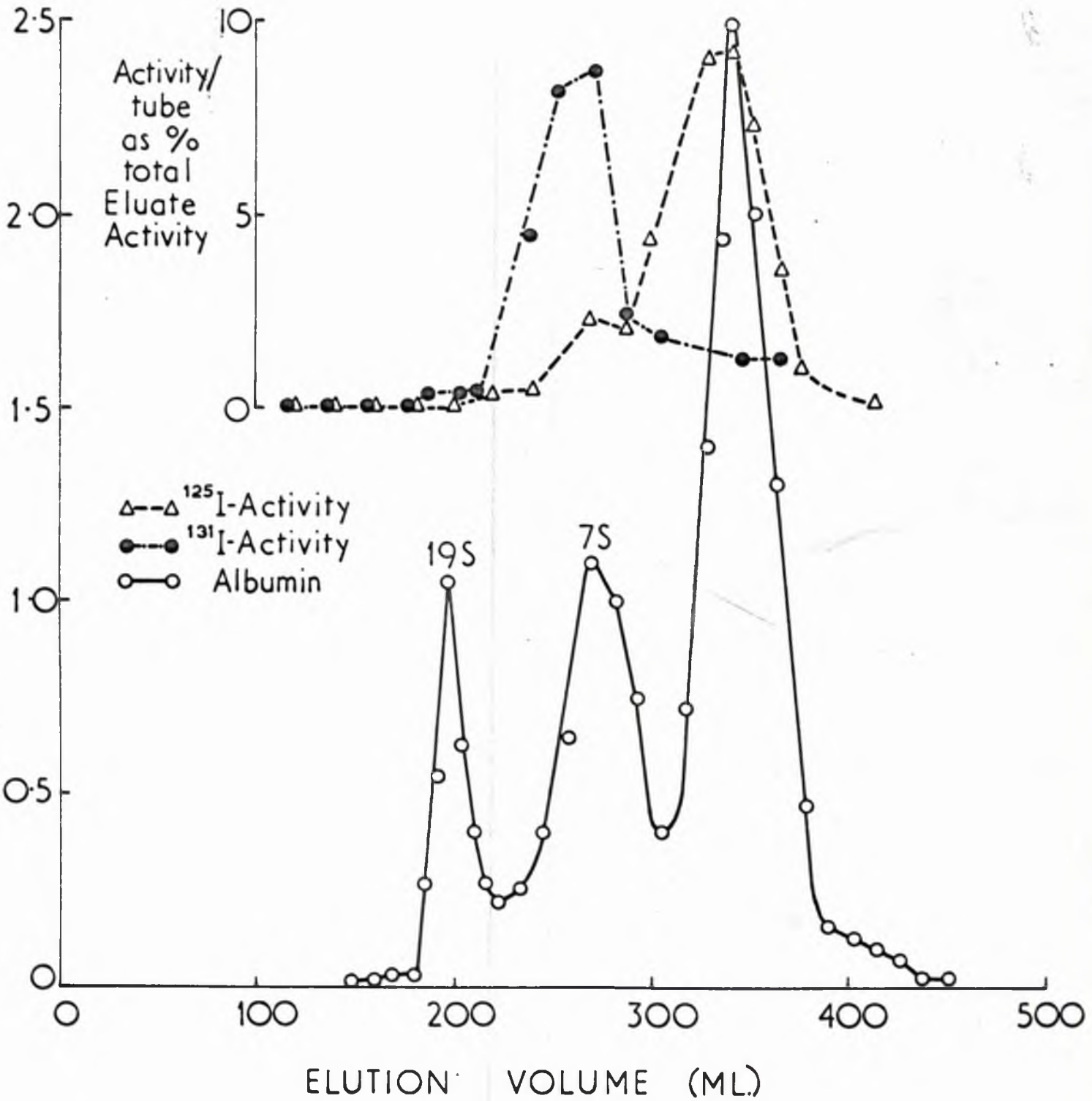
A carefully weighed amount (5 ml) of labelled gammaglobulin solution containing about 300 μc ^{131}I was injected into the marginal ear vein of each of the experimental animals. This was immediately followed by a known weight of albumin containing about 350 μc ^{125}I. Heparinised blood samples were collected, the first one 5 minutes after injection of the albumin and thereafter daily for 14 days. Standards were prepared by dilution of an aliquot of the labelled protein solutions used for injection.

(iv) Radioactivity Measurements

Count rates of samples of urine, faeces and plasma were determined twice, first on the 0.36 Mev photopeak of ^{131}I and then on the 0.035 Mev peak of ^{125}I. The ^{131}I "overlap" at the ^{125}I setting was taken into account by

FIGURE 9

PROTEIN and RADIOACTIVITY DISTRIBUTION FOLLOWING G-200 SEPHADEX GEL FILTRATION of ^{125}I LABELLED RABBIT ALBUMIN and ^{131}I LABELLED 7S GAMMA GLOBULIN ADDED to NORMAL RABBIT SERUM. COLUMN DIMENSIONS:-85×3cm BUFFER:-0·1MTRIS/HCl pH=8·0



application of a correction factor calculated from the count rate of the ^{131}I standard at its own setting relative to that at the ^{125}I setting. In this way, complete "separation" of the isotopes at their respective settings was achieved.

(v) Serum Protein Determinations

Total serum protein and individual fractions were determined as previously described. The protein concentration of the gammaglobulin band was taken as the concentration of IgG. Although this may not be strictly accurate, the error involved was probably not large because IgG forms by far the greatest part of the protein in the gammaglobulin band. Since, some of the other immune globulins are also included in this band, this over-estimation of IgG was probably counterbalanced by the fact that some IgG is found outside of the gammaglobulin band. The protein content of the gammaglobulin peak would therefore normally fairly well reflect the concentration of IgG in serum.

(vi) Immune Calculations

Because of some damage to the ears during injection of gamma-globulin, plasma volumes were determined from dilution of the labelled gammaglobulin, and this together with the appropriate serum concentrations enabled the intravascular pool of albumin and gammaglobulin to be calculated (CA and CG respectively). The determination of the extravascular pool was based on the extrapolation procedure (Sterling 1951), and these results verified for gammaglobulin by the "equilibrium-time method" of Campbell et al (1956).

Catabolic rates of the labelled proteins were assessed from the "apparent half-life" i.e. from the slope of the final exponential in the plasma activity curve (Sterling 1951) and by calculating the fraction of the intravascular pool degraded each 24 hours. This was determined "directly" from the radioactivity excreted every 24 hours divided by the total intravascular radioactivity (Campbell et al 1956) and "indirectly" by graphic analysis of the plasma activity curve (Matthews 1957).

RESULTS

(1) Albumin and Gammaglobulin Pool Sizes and Distribution Ratios in Normal and Fluke-Infected Rabbits

Four fluke-infected and three normal rabbits were used. Each infected rabbit had received 50 metacercariae 12 weeks previously and therefore had a burden of adult parasites at the time of the experiment. Each showed signs of infection in the form of a significant reduction in haematocrit, but serum protein differences were not significant (Appendix A). Albumin levels of the infected rabbits (2.49 gms % S.D. 0.39) were only slightly lower than those of the normal animals (2.99% gms. S.D. 0.60) whereas gammaglobulin concentrations were on average considerably greater (0.96 gms% S.D. 0.23 against 0.59 gms% S.D. 0.19 respectively).

^{131}I-labelled gammaglobulin and ^{125}I-labelled albumin were injected intravenously into each animal and plasma, urine and faeces collected and assayed for radioactivity. Plasma volume and the intra- and extravascular pools of each protein were estimated as previously described and distribution ratios calculated.

The mean plasma volume of the normal animals was 34.2 ml/kg (S.D. 3.8) while the corresponding figure for the infected group was 36.9 ml/kg (S.D. 2.8) thus confirming the finding of the previous experiment that no significant change in plasma volume was associated with infection. (The plasma volume of each animal is shown in Appendix A.) Albumin and gammaglobulin pool masses and distribution ratios calculated by extrapolation of the linear part of the plasma activity curve are shown in Table 13. Although there appeared to be a slight reduction in the size of the albumin pools no significant difference between the two groups of rabbits was apparent in the distribution of this protein. These findings were very similar to those of the previous experiment, but the reduction in albumin pool mass in the infected animals was barely noticeable possibly as a result of the lower burden of parasites.

A considerable increase in the size of the total gammaglobulin pool, mainly associated with an elevated intravascular mass was apparent in the infected animals compared to the controls. It was clearly only because of the small number of animals used in the experiment, and the degree of scatter, that this difference was not statistically significant. The distribution of this protein between compartments was very similar to that of albumin, but there was an indication of a slightly greater mass of gammaglobulin being present intravascularly relative to the total amount of this protein in the infected animals. Gammaglobulin pool sizes calculated by the equilibrium-time method were almost identical to those obtained by extrapolation (Appendix A). This method could not be applied to the

Turnover of ^{125}I-labelled Albumin / ^{131}I-labelled 7S Gammaglobulin

in Normal and Fluke-infected Rabbits

TABLE 13

DISTRIBUTION OF ALBUMIN AND GAMMAGLOBULIN (STERLING)

	Rabbit No.	Albumin					Gammaglobulin				
		CA (gm/kg)	EA (gm/kg)	TA (gm/kg)	EA/CA	EA/TA	CG (gm/kg)	EG (gm/kg)	TG (gm/kg)	EG/CG	EG/TG
CONTROL	429	1.17	1.99	3.16	1.70	0.63	0.28	0.35	0.61	1.33	0.57
	430	0.95	1.49	2.44	1.57	0.61	0.18	0.30	0.48	1.70	0.63
	433	0.91	1.62	2.53	1.78	0.64	0.16	0.21	0.37	1.27	0.56
	Mean	1.01	1.70	2.71	1.68	0.63	0.20	0.29	0.49	1.43	0.59
	S.D.	0.14	0.26	0.39	0.11	0.01	0.05	0.07	0.12	0.23	0.04
INFECTED	13	0.87	1.23	2.10	1.41	0.87	0.45	0.62	1.07	1.37	0.58
	23	0.80	1.36	2.16	1.70	0.63	0.41	0.41	0.82	1.00	0.50
	437	0.93	1.65	2.58	1.77	0.64	0.32	0.44	0.76	1.38	0.58
	313	1.04	1.85	2.74	1.78	0.68	0.23	0.35	0.58	1.50	0.60
	Mean	0.91	1.52	2.40	1.67	0.71	0.35	0.46	0.81	1.31	0.57
	P	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

estimation of the albumin pools since the ^{125}I injected activity was not known. It is unlikely however that the values obtained would have been significantly different from those shown in Table 13.

(ii) Catabolism of Labelled Albumin and Gammaglobulin

It is clear from the results in Table 14, that the degradation rate of both albumin and gammaglobulin was dramatically greater in the fluke-infected rabbits. This was shown by the shortened "apparent half-life" and also by the elevated values for the fractional catabolic rate. Albumin catabolic rates were very similar to those found previously and it is interesting to note that the turnover rate of immunoglobulin was higher than that for albumin even in the normal rabbits. The reason for this is unknown, although there is evidence that when more of the tracer is within or perhaps near the vascular space the fractional rate of degradation is greatest. In man, fibrinogen and macroglobulin which are almost entirely confined to the intravascular compartment have the highest fractional catabolic rates (Barth et al 1964; McFarlane, Todd and Cromwell 1964). In general the infected rabbits had considerably increased intravascular IgG pools and a rough correlation could be drawn between the mass of this pool and the catabolic rate of gammaglobulin in these animals.

The main site of gammaglobulin degradation isn't known, but it would appear from the plasma "clearance" figures obtained for the normal rabbits that the gastro-intestinal tract may play a more significant role in the normal degradation of this protein than in the catabolism of albumin.

Turnover of ^{125}I-labelled Albumin / ^{131}I-labelled 7S Gammaglobulin in Normal and Fluke-infected Rabbits

TABLE 14

CATABOLISM OF LABELLED PROTEINS

	Rabbit No.	^{125}I-labelled Albumin					^{131}I-labelled Gammaglobulin				
		$T_{\frac{1}{2}}$ (hours)	F(CA)	K*	Absolute Amount (gm/kg/day)	Faecal† Activity (ml. plasma)	$T_{\frac{1}{2}}$ (hours)	F(CG)	K*	Absolute Amount (gm/kg/day)	Faecal† Activity (ml. plasma)
CONTROL	429	134	0.203	0.201	0.237	0.69	96	0.298	0.276	0.078	0.92
	430	125	0.234	0.189	0.219	0.58	97	0.305	0.294	0.055	0.78
	433	119	0.259	0.228	0.236	0.47	95	0.282	0.280	0.045	0.85
	Mean	126	0.232	0.206	0.231	0.58	96	0.295	0.283	0.059	0.85
	S.D.	7.6	0.031	0.017	0.030	0.11	1.0	-	-	0.014	0.07
INFECTED	13	64	0.389	0.394	0.338	2.98	50	0.427	0.461	0.192	3.84
	23	58	0.483	0.462	0.386	3.43	48	0.485	0.523	0.199	3.49
	437	96	0.333	0.374	0.310	2.19	82	0.442	0.440	0.142	2.55
	313	112	0.335	0.368	0.349	1.32	84	0.452	0.412	0.104	2.00
	Mean	83	0.385	0.400	0.346	2.48	66	0.452	0.459	0.159	2.97
	S.D.	25.8	0.069	0.042	0.030	0.92	19.7	0.002	0.047	0.044	0.85
	P	< 0.05	< 0.02	< 0.001	< 0.002	< 0.02	< 0.05	< 0.001	< 0.002	< 0.02	< 0.01

* Fractional Catabolic Rate (Matthews)

† Mean 24-hour faecal radioactivity expressed as a "plasma clearance"

It should however be pointed out that these results could also be explained by an increased ability of the gut to catabolise albumin because of its greater solubility and thereby effect more complete absorption of its breakdown products. Since radioiodide may also appear in the faeces by passage into the intestinal tract via saliva and intestinal juices, no conclusive statement may be made concerning the relative amounts of these proteins normally degraded in the lumen of the intestinal tract. It is apparent however that in the fluke-infected rabbits significant amounts of both isotopes were excreted in the faeces and the most likely explanation of the higher faecal "clearances" obtained is that a considerable amount of immunoglobulin in addition to albumin had passed into the alimentary tract.

DISCUSSION

The results of these studies show that really no useful information on the effects of liver-fluke disease on plasma protein metabolism can be obtained by the application of conventional analytical techniques. Infected animals were hypoalbuminaemic but estimates of pool sizes and distribution ratios indicated that despite a reduction in the total mass of albumin the intravascular pool was not significantly affected (Table 15). One might therefore be tempted to think that in these animals the plasma albumin picture was only very slightly affected by infection with liver-fluke. However from the "kinetic" part of the experiment it is apparent that this was not the case and that the infected animals were suffering from a hypercatabolic hypoalbuminaemia. It has been suggested in the past that interference with albumin synthesis due to liver damage might be a contributory factor in the hypoalbuminaemia of fascioliasis (Thorpe 1965; Sinclair 1962; 1968). This certainly cannot be true of the 10-week infection in rabbits. The finding of a reasonably normal intravascular pool mass can be explained either by an increased albumin synthesis or a greater net transfer of extravascular albumin to the circulation. Since on average about twice as much albumin was broken down intravascularly each day in the infected rabbits, the extravascular pool would have been completely drained very quickly if the synthesis rate of albumin had remained constant. This did not occur and it was clearly impossible that albumin lost from the circulation was compensated for by a "tap" of the extravascular pool. The most likely

MEAN ALBUMIN POOL SIZES AND CATABOLIC RATES IN NORMAL AND FLUKE-INFECTED RABBITS

TABLE 15

Rabbit No.	Plasma Volume (ml/kg)	Albumin Distribution				Albumin Catabolism			
		CA (gm/kg)	EA (gm/kg)	TA (gm/kg)	$T_{\frac{1}{2}}$ (hours)	F(CA)	K*	Absolute Amount (gm/kg/day)	Faecal† Activity (ml.plasma)
Control	37.4	1.26	1.73	2.99	211	0.195	0.191	0.242	0.98
S.D.	4.1	0.26	0.31	0.47	44	0.020	0.051	0.039	0.52
Infected	38.1	0.99	1.38	2.37	81	0.432	0.434	0.405	2.72
S.D.	2.0	0.14	0.27	0.38	17	0.084	0.072	0.150	0.80
P	N.S.	N.S.	<0.05	<0.02	<0.002	<0.002	<0.002	<0.02	<0.02

* Fractional Catabolic Rate (Matthews)

† Mean 24-hour faecal radioactivity expressed as a"plasma clearance".

hypothesis is that the intravascular pool was kept within normal limits by a marked increase in synthesis, although the finding of a reduced extravascular pool indicates that synthesis did not quite keep pace with catabolism.

It is however probable that during migration through the liver, damage to hepatic cells may have been sufficiently extensive to cause some reduction in albumin synthesis. Jennings et al (1960) noted increased levels of serum alkaline phosphatase and S.G.O.T. in fluke-infected sheep during this time. These authors were careful to point out that after the flukes had become established within the bile ducts of the host, the serum levels of these enzymes returned to normal while albumin concentrations continued to decline, indicating that impaired liver function was not the cause of the hypoalbuminaemia once the infection was patent.

The mechanism by which albumin synthesis is stimulated is not known, but it would not be unreasonable to assume that when hypercatabolism commenced, i.e. when the flukes first entered the bile ducts of the host (Dargie, Section 3), the albumin lost from the circulation was replaced from the extravascular pool, and as this pool was reduced a mechanism "triggering" increased synthesis came into play to balance this effect. Since albumin is synthesised entirely by the liver, increased synthesis would have resulted from an increased albumin production per cell or by a greater number of cells producing albumin or both. The relative

importance of these factors is difficult to evaluate but the evidence to date suggests that liver regeneration is probably the more important. This evidence is twofold. Firstly, severe forms of nephrosis in which the livers are producing albumin at nearly twice the normal rate is characterised by a marked enlargement of the liver above normal. Increased liver weight is also associated with fascioliasis and Singlair (1964) has shown a fairly close direct correlation between the number of parasites present and liver weight, indicating that within limits the degree of liver damage may determine the amount of regeneration and hence increase in albumin synthesis. In addition, Glinos and Gey (1952) have shown that plasmapheresis causes mitosis in livers of normal rats, and more recently Glinos (1956 (a) and (b)) has produced evidence that the regenerative changes after partial hepatectomy can be inhibited by increasing the plasma concentration of albumin. This author has therefore suggested that the plasma albumin concentration controls regeneration. Although marked reduction in serum albumin level does not usually occur until the flukes have become established within the bile ducts, it is probable that subtle changes in the albumin concentration in the immediate environment of the hepatic cells may occur earlier and independently of a change in the plasma. For instance a time lag is known to occur between albumin synthesis and its appearance extracellularly (Green and Anker 1955). It is possible that this delay depends on the protein concentration gradient between the microsomes, hepatic interstitial fluid and plasma.

The extent to which albumin synthesis can be increased is not known but the evidence presented here suggests that since production did not quite keep pace with catabolism, it may have been at most doubled. Similarly, in human pathology a marked compensatory increase in albumin production is rare and in gastro-intestinal protein loss the rate of synthesis seldom exceeds twice the normal average (Gordon, Bartter and Waldmann 1959; Schwartz and Jarnum 1959 and 1961).

On the other hand, in the infected animals, IgG synthesis must have been increased by three of four fold to account for the greater body pools of this protein despite the marked hypercatabolism. These findings are in agreement with those obtained from turnover studies on human diseases characterised by hypergammaglobulinaemia and confirm that the synthesis capacity of the plasma proteins can be increased in pathological conditions to a remarkably different extent (Birke, Liljedahl, Olhagen, Plantin, Ahlinder 1963; Andersen 1964).

However, hypercatabolism or increased loss <u>per se</u> do not stimulate production of IgG i.e. although albumin synthesis was increased in response to a low serum level of this protein, reduction in the intravascular IgG content produces no increase in IgG synthesis. For example, it has been shown that loss of IgG into the intestinal tract does not stimulate synthesis (Ahlinder, Birke, Liljedahl, Plantin 1964; Waldmann and Schwab 1965) and serum gammaglobulin levels are determined only by the degree of antigenic stimulus. The raised gammaglobulin

concentrations observed in fascioliasis which usually become apparent just after the flukes enter the liver are most likely produced by the hepatic mesenchymal cells in response to the parasite, although the importance of the part played by extrahepatic tissue in this respect is unknown. Jabbari and Levy (1967) have shown that ^{14}C-labelled lysine is incorporated into serum gammaglobulin to a much greater extent in cirrhotic than normal perfused rat livers and indicated that the increased serum levels of gammaglobulin and mesenchymal cell replication were related. It is therefore likely that the liver in addition to being responsible for the increased albumin production associated with the disease, also responds to damage by an increased immunoglobulin synthesis. Sinclair (1963) noted that the characteristic rise in serum globulin levels in lambs following infection with F. hepatica did not occur when corticosteroid was administered. He also noted accelerated growth of the parasite in such animals suggesting that although antibody production did not stop the flukes from entering the bile ducts it did play some part in slowing their progress through liver tissue.

When the flukes enter the bile ducts serum gammaglobulin levels although still elevated above normal do not generally continue to rise, presumably because a compensatory increase in catabolism occurs at this stage of infection.

The results of faecal excretion of albumin, gammaglobulin and P.V.P. suggest that the increased catabolism is due to a direct loss of these proteins into the gastrointestinal tract, presumably via flukes and

bile. Assuming the hypercatabolism to be due entirely to increased enteric protein loss, the magnitude of this leak was equivalent to about 25 ml. of plasma per day or 25% of the plasma volume. Such a loss of plasma proteins also occurs in fluke-infected sheep (Holmes 1969), and probably in sufficient amounts to account for the reduced calcium and magnesium levels reported in such animals by Sinclair (1962).

Although plasma proteins leaking to the gut will not be completely lost to the animal, it will nevertheless place a heavy demand on the synthetic mechanisms since the infected animal will again have to resynthesise the protein from reabsorbed amino acids. However attention must be given not only to the nutritional but also the immunological consequences of gastro-intestinal protein leakage. The increased degradation of immune globulins which occurs in the gastro-intestinal tract may bring about significant impairment of protective mechanisms since in the case of these proteins specific antibody function will have been lost.

The mechanism by which plasma constituents leak into the gut is not known, but there can be little doubt that transfer could only occur through the bile duct epithelium, either as a result of the mechanical damage caused by the flukes or as a result of the parasites blood sucking activities. This will be discussed later in greater detail when further results are considered.

It must be made clear that all the experiments described in this section were carried out on rabbits with ten-to-twelve week infections,

i.e. they were harbouring populations of adult parasites. It will be necessary to study protein turnover on animals during the early stages of the disease, and also animals with long-standing infections to determine the possibility of a further increment in catabolism due to the presence of larger flukes within the bile ducts.

SUMMARY

(1) Serum albumin labelled with ^{131}I was used to measure the total body pool, distribution and catabolic rate of albumin in four rabbits infected with F. hepatica and four control rabbits. The fractional catabolic rate of albumin in the infected rabbits was strikingly greater than that in the controls, suggesting hypercatabolism as an important factor in the hypoalbuminaemia associated with F. hepatica infections. The result is consistent with a substantial loss of albumin into the gastrointestinal tract presumably in the bile.

(2) Confirmation of a significant transfer of plasma macromolecules into the gut in infected rabbits was obtained from studies on the faecal excretion of labelled polyvinylpyrrolidone.

(3) To study whether globulin was being catabolised at a similar rate to albumin in fluke-infected rabbits, a simultaneous double labelling experiment was carried out in which albumin was trace-labelled with ^{125}I and 7S-gammaglobulin fraction with ^{131}I. These were injected into three normal and four infected rabbits. In the fluke-infected animals the

hypercatabolism of albumin was accompanied by similar hypercatabolism of 7S-immunoglobulin. This was most easily explained by loss into the digestive tract.

SECTION 2

THE ANAEMIA OF FASCIOLIASIS IN THE RABBIT: STUDIES WITH ^{51}Cr AND ^{59}Fe-LABELLED RED CELLS

INTRODUCTION

Anaemia is defined as a reduction in the circulation of either haemoglobin or erythrocytes, and like hypoproteinaemia is caused by one or a combination of factors, which may effect a disturbance in the balance which normally exists between synthesis and catabolism. It is usual to classify anaemia according to aetiology and few topics in parasitology have aroused as much controversy as the cause of the anaemia associated with chronic fascioliasis. Although many theories have been advanced, until fairly recent times most have been based on rather thin evidence.

Perhaps the most popular theory is that blood loss brought about by the feeding activities of the flukes is the primary factor in causing the anaemia. Weinland and Von Brand (1926) and Stephenson (1947) demonstrated that flukes could ingest blood *in vitro*. The latter author as a result of histological and spectroscopical examination of the dark caecal contents of the flukes showed that this material was degraded haemoglobin and concluded that the adult fluke was haematophagic. These findings have since been supported by others (Van Grembergen 1950, Urquhart 1955, Todd and Ross 1966, Symons and Boray 1967). However, Dawes (1963(a) and (b)) did not consider that the adult fluke is haematophagic. On the basis of histological examination of the infected bile-ducts, he concluded that the flukes feed on hyperplastic biliary mucosa, but made no attempt to explain how the anaemia could arise.

Blood loss, and hence anaemia, may also result from an increased intravascular degradation of erythrocytes, and several authors have

suggested that the liver fluke secretes a haemolytic toxin. Guerini (1908) demonstrated two types of secretory cells in the cuticulum of F. hepatica and suggested that the secretions of these cells may be absorbed and may destroy the red cells, while Marcone (1940) claimed that the sera of fluke-infected sheep could haemolyse normal red cells. It seems strange that on the basis of such thin evidence each of the standard text books on veterinary helminthology refers to the production of a haemolytic toxin by the parasite (Monnig 1950; Cameron 1951; Lapage 1962).

In addition to an increased rate of destruction of erythrocytes, anaemia may develop because of impaired synthesis. Basically two factors may be responsible for a reduction in erythrocyte production.

In the first place, production may be inadequate because of a deficiency of one or several of the factors necessary for erythropoiesis, e.g. vitamin B_{12}, iron etc. In these conditions, both the number and the quality of the red cells are reduced despite a hypercellular marrow. Balian (1964) suggested that a deficiency of the anti-anaemic principle of the liver could account for the anaemia and Obara, Sonoda and Watanabe (1940) and Sewell (1967) studying the effects of F. gigantica infections in rabbits and cattle respectively concluded that the parasite may preferentially absorb vitamin B_{12}.

Sinclair (1964) also considered the anaemia to be of the dyshaemopoietic type and suggested that it may be caused by the disturbance in protein metabolism which accompanies F. hepatica infections. He also

indicated on the basis of the rate of clearance of intravenously injected ^{59}Fe from the plasma and its subsequent incorporation into circulating red cells that his fluke-infected sheep may have been iron deficient. However, in a later study, Sinclair (1965) demonstrated the continued presence of marrow haemosiderin and a low utilisation of injected radioiron in fluke-infected sheep, and concluded that although some disturbance of iron metabolism does occur in fascioliasis, iron deficiency is not the primary cause of the anaemia. This author suggests that because of the rise in serum globulin levels in infected animals, and the known function of the reticuloendothelial system of destroying senescent and non-viable erythrocytes, the hyperactive reticuloendothelial system may bring about an increased destruction of red cells. Recently, Sinclair (1967) has postulated the release of an "unknown toxic substance" by the fluke which depresses erythropoiesis presumably by its direct action on the bone marrow. No experimental evidence was cited to substantiate this phenomenon. It is thus apparent that almost every conceivable mechanism has been put forward to account for the anaemia associated with the disease.

Although characterisation of anaemia solely on the basis of the causative factor is useful, it is somewhat ambiguous because various aspects of the disease may be considered as "causes". For example, anaemia "caused by" blood loss may itself involve several "causes" such as increased haemolysis or haemorrhage, and furthermore, these factors may be related. Thus chronic blood loss may result in a deficiency of one

or more of the materials required for red cell construction, and as a result, erythrocytes are produced which are more susceptible to haemolysis. It is therefore apparent, that although haemorrhage may be regarded as the primary "cause" of an anaemia, other factors may later come into play which will render diagnosis of the "cause" more difficult.

For this reason, anaemia should, in addition to aetiology, be classified according to its morphological characteristics. Such a classification is usually helpful in diagnosis because the characterisation of anaemia according to the size and haemoglobin content of the erythrocyte directs the future investigation towards a definite group of possible causative factors and eliminates others from consideration.

Unfortunately, the morphological characteristics of the anaemia associated with fascioliasis are also disputed. Urquhart (1955) and Thorpe (1963) demonstrated that the anaemia of infected rabbits and rats was similar to that produced by repeated bleedings. These authors characterised the anaemia as hypochromic and macrocytic, with reticulocytes present in the peripheral circulation. On the other hand, a normochromic, normocytic anaemia has usually been associated with experimental chronic ovine fascioliasis (Sinclair 1962; Sewell, Hammond and Dinning 1963). However following extensive field trials Jennings and Armour (1969) have demonstrated that in lambs the anaemia is initially normochromic and normocytic, but as infections become longer established, it subsequently develops into a type characterised by hypochromia, macrocytosis and marked reticulocytosis.

It is clear that classification of the anaemia in this way is not entirely satisfactory either because the same disease may produce more than one type of anaemia. For example, chronic blood loss may produce a simple microcytic type of anaemia at one stage, and a hypochromic microcytic type at another. However, although each classification has shortcomings when used alone, both are very helpful when used together. In this section of the thesis evidence is presented based on the use of ^{51}Cr and ^{59}Fe-labelled red cells that the primary cause of the anaemia is blood loss and that this provides a rational explanation for the observed differences between infected and normal animals in the morphological characteristics of the peripheral blood.

Isotopically labelled red cells provide a useful marker for studying the turnover of the erythrocyte *in vivo*, but rather surprisingly few reports have been published on the use of such labels for studying the anaemia of fascioliasis. Jennings *et al* (1956) first used tagged erythrocytes to measure the quantity of blood lost in the anaemia of *F. hepatica* infections in rabbits. These authors injected ^{32}P-labelled red cells into fluke-infected and control animals and related the radioactivity found in the flukes after one hour with that of the circulating blood. A figure of 0.2ml. was calculated as the amount of blood consumed by each fluke per day.

Although cells labelled with ^{32}P can be used for short-term experiments of this nature, they are of little use for long-term studies because of the rapid loss of the isotope from the cells. The suitability

of a label for following the fate of the red cell is governed by the same conditions as previously applied to the labels used for estimation of the magnitude of protein transfer into the gastrointestinal tract viz. it should be firmly attached to the red cell without altering its life-span, and following senescence or destruction of the cell, the isotope should not be reutilised, but should be quantitatively excreted in the urine if degraded intravascularly, and in the faeces if passed into the alimentary canal as a result of enteric blood loss. Although no label has yet been found to satisfy completely all these conditions, ^{51}Cr fulfills most.

Gray and Sterling (1950(a)) first demonstrated that a firm union between erythrocytes and chromium was rapidly established *in vitro* with no apparent damage to the cells, and subsequently used the technique for the estimation of circulating red cell volume (Gray and Sterling 1950(b)). When ^{51}Cr-labelled erythrocytes are broken down in the circulation, the isotope is excreted rapidly and quantitatively in the urine (Ebaugh, Emerson and Ross 1953; Roche *et al* 1957; Jennings 1962). The method has therefore been widely employed for red cell survival studies in man (Ebaugh *et al* 1953; Mollison and Veall 1955), and domestic animals (Donohue, Motulsky, Giblett, Pirzio-Biroli, Vivanuvatta and Finch 1955; Stohlman and Schneiderman 1956; Bush, Jensen, Athens, Ashenbrucker, Cartwright and Wintrobe 1956; Belcher and Harriss 1959).

In all cases, however, it has been found that the rate of disappearance of ^{51}Cr-labelled red cells from the circulation is considerably

greater than the known rate of disappearance of non-labelled cells as determined by differential agglutination. This is because ^{51}Cr survival curves are the resultant of at least two processes - loss of red cells by senescence, and elution of ^{51}Cr from intact red cells. Recent work on cattle and sheep suggests that elution occurs in two phases - a short period of rapid elution over the first 24 hours following injection during which time as much as 30 - 50% of the injected activity is excreted, followed by a slower exponential loss (Todd and Ross 1966; McSherry, Van Dreumal and Robinson 1966; Drury and Tucker 1958; Holmes 1969). In man, an initial loss of 10% of the injected activity has been observed (Mollison and Veall 1955) while in the rabbit Jennings (1962) noted a 20% reduction in circulating red cell activity 24 hours following injection.

Various formulae have been derived for correcting ^{51}Cr-labelled red cell disappearance curves for elution in order that red cell life-span may be estimated by comparing the survival estimated by differential agglutination with the ^{51}Cr survival.

In practice conversion for elution is hardly worth while provided it is assumed that the elution rate of chromium from the red cell is constant under normal and pathological conditions. The validity of this assumption has recently been questioned. For instance, several groups of workers have suggested that hypochromic (iron-deficient) red cells are more subject to haemolysis than normal erythrocytes (Kaiderling, Schmidt and Lee 1957; Layrisse, Linares and Roche 1965; Loria,

Sanchez-Medal, Lisker, de Rodriguez and Labardini 1967). These authors have further shown (by differential agglutination) that normal cells survive normally in the circulation of patients with iron-deficiency anaemia, whereas the erythrocytes of iron-deficient subjects transfused into normal recipients show a reduction in survival.

It should be made clear however, that although all types of anaemia have been reported to involve excess haemolysis, the importance of this in decreasing the level of circulating haemoglobin varies. In the so-called haemolytic anaemias it is the major aetiological factor, whereas in iron-deficiency anaemia caused for example by hookworm infection, it is the result of the anaemic process, rather than its cause, and it is doubtful if such haemolysis contributes significantly to the anaemia since the iron released by red cell destruction will be quickly reutilised.

In any case, excess haemolysis may be easily detected using ^{51}Cr-labelled red cells since intravascular degradation of this label results in the appearance of an abnormally large amount of the isotope in the urine. Thus in studying the aetiology of anaemia using this label excess haemolysis may be safely ignored provided the urinary excretion of isotope is similar in anaemic and normal animals.

Perhaps the greatest advantage of ^{51}Cr-labelled red cells is that where haemorrhage occurs into the gastro-intestinal tract the isotope is excreted quantitatively in the faeces. This has been adequately demonstrated in man, dog and rabbit, although as previously discussed,

small amounts of the isotope can be absorbed from the intestinal tract of sheep following oral administration (Clark et al 1962).

From these studies, it is apparent that if intravascular degradation occurs, most of the radioactivity will appear in the urine, whereas if the anaemia involves haemorrhage into the gut then the ^{51}Cr will be excreted in the faeces. Thus ^{51}Cr-labelled erythrocytes provide a very useful tool for studying the behaviour and fate of erythrocytes in vivo.

Jennings (1962) first used ^{51}Cr-labelled red cells to study the anaemia of fascioliasis in the rabbit. In a small pilot experiment involving two normal and an infected rabbit, this author noted that there was an abnormally fast rate of disappearance of ^{51}Cr-labelled red cells from the circulation of the infected rabbit relative to the normal animals. Whereas no difference was found in the amount of radioactivity excreted in the urine, a markedly increased faecal excretion of isotope was noted in the infected rabbit. Jennings concluded that the anaemia was not due to intravascular haemolysis but to a loss of blood via the bile to the gut.

The validity of the conclusion reached by Jennings as a result of this work is only diminished by the size of the experiment. Obviously too much emphasis cannot be placed on the results obtained from one fluke-infected rabbit. It was therefore decided to repeat this work on a larger group of animals and to determine whether the degree of anaemia and magnitude of the blood loss bore any simple relationship to the number of flukes present within the bile ducts.

The rate of disappearance of ^{51}Cr-labelled red cells from the circulation not only reflects loss of labelled erythrocytes due to intravascular breakdown and haemorrhage but also the rate at which new unlabelled cells are added to those labelled cells already circulating. Although Jennings concluded that the faster rate of disappearance of ^{51}Cr-labelled erythrocytes from the circulation of the infected rabbit was associated with considerable loss of red cells into the gut, his results did not indicate if this loss was compensated for by increased erythropoiesis. This information can be derived (albeit indirectly) from ^{51}Cr-labelled red cell survival curves only if the circulating red cell volume of the animal is determined at the beginning and again at the end of the study. For instance, if an animal lost the equivalent of 12% of its blood volume per day into the intestinal tract (this was the figure quoted by Jennings for his fluke-infected rabbit) then it would be expected that some reduction in red cell volume would result over the experimental period. If however the red cell volume was maintained in the face of such a loss, it would be reasonable to conclude that erythropoiesis must have been considerably increased to meet this continuous drain.

Studies using ^{51}Cr-labelled red cells were therefore undertaken not only to measure the magnitude of the blood loss suffered by fluke-infected rabbits, but also to assess the ability of these animals to increase their rate of red cell production when subjected to chronic blood loss.

Although certain conclusions concerning erythropoiesis may be derived from the rate of disappearance of ^{51}Cr-labelled erythrocytes from the circulation, this technique cannot be used to measure quantitatively the rate of red cell production. This can only be done using a label which is incorporated directly into the haemoglobin molecule.

Since the introduction of radioiron of high specific activity, measurement of the rate of disappearance of an intravenously injected dose of ^{59}Fe from the plasma, and its subsequent appearance in the circulating red cells has become widely accepted as a measure of erythropoiesis.

Iron circulates in the plasma as a complex with transferrin, and although the normal plasma iron level is only about 120 µgm/100 ml. i.e. about 0.1% of the total body iron) it is nevertheless of vital importance since it represents the sole means by which iron can be transported from one part of the body to another. Since only negligible quantities of iron are normally lost from the body, the level of plasma iron represents the dynamic equilibrium between iron supplied from the gut and body stores, and that being removed by the bone marrow and other tissues.

Most of the iron leaving the plasma at any one time is being carried to the marrow for haemoglobin synthesis, and it is therefore possible with a knowledge of the rate of clearance of intravenously injected ^{59}Fe from the plasma and of the level of iron in the plasma at that time to obtain some measure of the amount of iron being turned over through the plasma and hence the rate of red cell production (Huff,

Hennessey, Austin, Garcia, Roberts and Lawrence 1950; Bothwell, Hurtado, Donohue and Finch 1957).

In the past, measurement of the radioiron clearance rate alone, without regard to serum iron level has been used to characterise erythropoiesis. Although the clearance rate is accelerated with increased erythropoiesis, and reduced in conditions associated with decreased red cell production, it is so dependent on the level of plasma iron that any measurement of either iron level or clearance rate alone has little meaning. For instance although the normal diurnal variation in plasma iron level may alter the radioiron clearance rate, they do not significantly affect the absolute turnover rate, and even in patients with iron storage disease (haemochromatosis) who have a 20-fold increase in iron stores above normal, radioiron utilisation is depressed, and the absolute plasma iron turnover rate is only slightly greater than normal (Bothwell et al 1957). These authors have further shown that increased red cell destruction without increased production brought about by injecting damaged erythrocytes does not result in an increased plasma iron turnover, despite marked elevations in serum iron levels. These findings suggest that the absolute turnover rate is normally quite precisely regulated.

On the other hand, marked variations result from altered erythroid marrow activity. Decreased marrow activity induced by irradiation results in a significant reduction in plasma iron turnover, whereas if the marrow is activated by plebotomy a prompt increase in plasma iron turnover occurs (Bothwell et al 1957). Thus it is now generally accepted that

erythroid marrow activity is the dominant factor in determining plasma iron turnover.

The technique may therefore be used to assess the relative importance in the aetiology of anaemia of reduced red cell production, resulting either from bone marrow failure or nutritional deficiency, and increased loss. For example anaemia associated with bone marrow failure (aplastic or hypoplastic anaemia) is usually characterised by an elevated serum iron and a very slow rate of clearance of radioiron from the plasma. In addition, only a very small % of the injected activity utlimately appears in the red cells. On the other hand, refractory anaemia associated with a hyperplastic marrow (pernicious and nutritional macrocytic anaemia) is characterised by a higher than normal serum iron and a rapid ^{59}Fe plasma clearance. The plasma iron turnover rates are therefore elevated but radioactivity is slow to appear in the circulating erythrocytes.

Marrow hyperplasia is also a common feature of anaemia caused by increased breakdown of red cells, e.g. haemolytic and haemorrhagic anaemia. The presence of a greater than normal number of red cell precursors is accompanied by a rapid iron clearance, and it ismot unusual for the marrow to attain its maximum uptake within one or two hours after injection of the ^{59}Fe. Thus the red cell ^{59}Fe activity increases rapidly to a maximum, but thereafter declines also at a markedly increased rate. Thus although the plasma iron turnover rate is not influenced by increased red cell destruction <u>per se</u> it is markedly elevated when breakdown is

compensated for by an increased marrow activity.

In order to measure erythropoiesis and hence to assess the importance of dyshaemopoiesis as being a causative factor in the anaemia of fascioliasis, ferrokinetic studies were carried out on normal and fluke-infected rabbits. It was hoped that this technique would extend our understanding of red cell production and iron metabolism in these parasitised animals and in conjunction with the results of the ^{51}Cr-labelled anaemia enable a rational explanation for the anaemia of fascioliasis.

Whereas studies with ^{51}Cr-labelled red cells may be used to measure fairly accurately the amount of blood which passes into the gut, the technique suffers from the additional disadvantage that no information is obtained regarding possible reabsorption of the various blood elements from the lumen. Such information is obviously essential in order that the importance of nutritional factors in increasing the severity of the anaemia may be assessed. Because of the possibility that passage of blood into the alimentary tract was of sufficient magnitude to cause the anaemia associated with fascioliasis and the known development of iron deficiency as a secondary factor in anaemia caused by chronic blood loss, it was considered important to measure the extent to which these animals might be able to reutilise iron which passed into the gut.

Two quite different approaches were used to study iron absorption. The first of these involved following simultaneously the disappearance of

^{59}Fe and ^{51}Cr-labelled erythrocytes from the circulation, and determining the blood and hence iron loss from the faecal excretion of both isotopes according to the method of Roche, Perez-Gimenez and Levy 1957. Faecal radioactivity from ^{51}Cr was taken to indicate the amount of blood lost into the intestine, and from the blood haemoglobin concentration intestinal iron loss was then calculated. On the other hand radioactivity from ^{59}Fe indicated the actual amount of iron lost in the faeces and by subtraction, the amount of iron reabsorbed was calculated.

The second experiment involved the faecal recovery of ^{59}Fe and ^{51}Cr after the administration of oral doses of lysed doubly labelled red cells. Absorption was calculated as the difference between the total radioactivity administered and that recovered in the faeces.

MATERIALS AND METHODS

(i) Experimental Animals

Each of the infected rabbits used in these studies was infected with either 100 or 50 metacercariae three months previously and was showing signs of infection in the form of reduced haematocrits and an alteration in serum albumin/globulin ratio.

(ii) Haematological Methods

Venous blood haematocrit was measured by the capillary micro-method and red cell counts using a Coulter counter. Blood haemoglobin was estimated by the cyanmethaemoglobin procedure (Van Kampen and Zijlstra 1961) and serum iron by the method of Ramsey (1957).

(iii) Plasma Volumes

These were measured by the intravenous injection of ^{125}I-labelled rabbit serum albumin and application of the dilution principle.

(iv) Plasma and Red Cell ^{59}Iron Labelling

A measured volume of sterile ^{59}Ferric citrate (specific activity 8μc/μg) was adjusted to give radioactivity of 50 μc and injected into the marginal ear vein of each of the infected and normal rabbits. Heparinised blood samples were taken at suitable intervals from the opposite ear vein and analysed for radioactivity as described below. A standard solution was prepared from a measured volume of the injected solution.

(v) Labelling of Red Cells with ^{51}Cr

Heparinised samples of rabbit blood were treated with ^{51}Cr as sodium chromate and incubated at room temperature. The labelled cells were

then washed with 0.9 % NaCl until free of unbound ^{51}Cr and suspended in 0.9% NaCl for injection.

(vi) Injection of ^{51}Cr-labelled Cells and Blood Sampling

A measured volume of labelled cell suspension adjusted to give radioactivity of 100 μc was injected into the marginal ear vein, each rabbit receiving its own labelled red cells. Blood samples were subsequently collected in heparinised tubes, the first one 5 minutes after injection, then twice daily for 5 days and thereafter daily for a further period of 3 days. Standard solutions were prepared from each of the labelled cell suspensions by diluting an appropriate aliquot with 0.01 N NaOH.

(vii) Preparation of ^{59}Fe / ^{51}Cr-Labelled Red Cells

Erythrocytes containing ^{59}Fe-labelled haemoglobin were obtained by cardiac puncture from rabbits 10 days after intravenous injection of ^{59}Fe citrate. The cells were then labelled with ^{51}Cr as previously described.

(viii) Collection of Urine and Faeces

The total output of urine and faeces for each 24-hour collection period was measured and in most cases suitable samples taken in duplicate for radioactivity determination. However, the radioactivity of the total faecal collection was determined in the experiment involving oral administration of doubly labelled cells.

(ix) Radioactivity Measurement

Standard aliquots of blood, plasma and the 24-hour urine collection

were pipetted into counting tubes and made up to a volume of 5 ml. Faecal samples were similarly packed to 5 ml. for counting. Because of differences in the energies of their radiations, ^{59}Fe and ^{51}Cr can be separately determined in the same sample. Suitable aliquots of standard solutions of these isotopes were assayed at regular intervals, corrections for radioactive decay being based on the activities of these solutions.

RESULTS

(A) STUDIES WITH ^{51}Cr-LABELLED RED CELLS

Four fluke-infected rabbits (100 metacercariae each 12 weeks previously) and 4 control rabbits were each injected with autologous red cells labelled with ^{51}Cr. The persistence of labelled cells in the circulation and the urinary and faecal output of isotope were followed over a period of 8 days.

(1) Red Cell and Blood Volume Determination

Following injection of the labelled erythrocytes 5 minutes were allowed for complete mixing within the circulation, after which time the first blood sample was withdrawn. The radioactivity of a carefully measured 1 ml. aliquot of this sample was divided into the injected activity to obtain the blood volume. By use of the venous haematocrit (determined at the time of collection) the activity of this 1 ml. sample of whole blood was converted to radioactivity per ml. of packed red cells thus:

$$\text{Counts/sec/ml. packed red cells} = \frac{\text{Counts/sec/ml. whole blood} \times 100}{\text{Venous Haematocrit}}$$

and the circulating red cell volume of each animal calculated by dividing the value obtained into the injected activity. Absolute values in all cases were expressed on a body weight basis and the results obtained shown in Table 16.

Although the circulating red cell volumes of the two groups were very similar, a slight (though barely significant) increase in blood volume was noted in infected rabbits relative to the controls, indicating some possible expansion of plasma volume in these animals. Blood and red cell volumes were re-determined at the end of the study in order to check that no change had occurred during the course of the experiment. The mean blood volume of the infected animals at this time was 47.8 ml/kg (S.D. 9.1) while that of the controls was 39.2 ml/kg (S.D. 4.5). This difference was not statistically significant ($P > 0.1$).

(ii) Persistance of Labelled Cells in Fluke-infected and Normal Rabbits

The radioactivity of each blood sample was expressed as a % of the equilibrium 5 minute post-injection value and from the venous haematocrit determinations on each sample the activity per ml. of red cells was then calculated as previously described. A semi-logarithmic plot of both whole blood and red cell activity against time was made. Red cell activity curves obtained for one of the normal and one of the fluke-infected rabbits are shown in Figure 10. It is apparent that the rate of disappearance of the labelled cells from the circulation of the fluke-infected rabbit was considerably greater than that of the normal animal. This rate was expressed as a "half-life", values for which are

^{51}Cr Labelled Erythrocyte Turnover Studies in Normal and Fluke-infected Rabbits

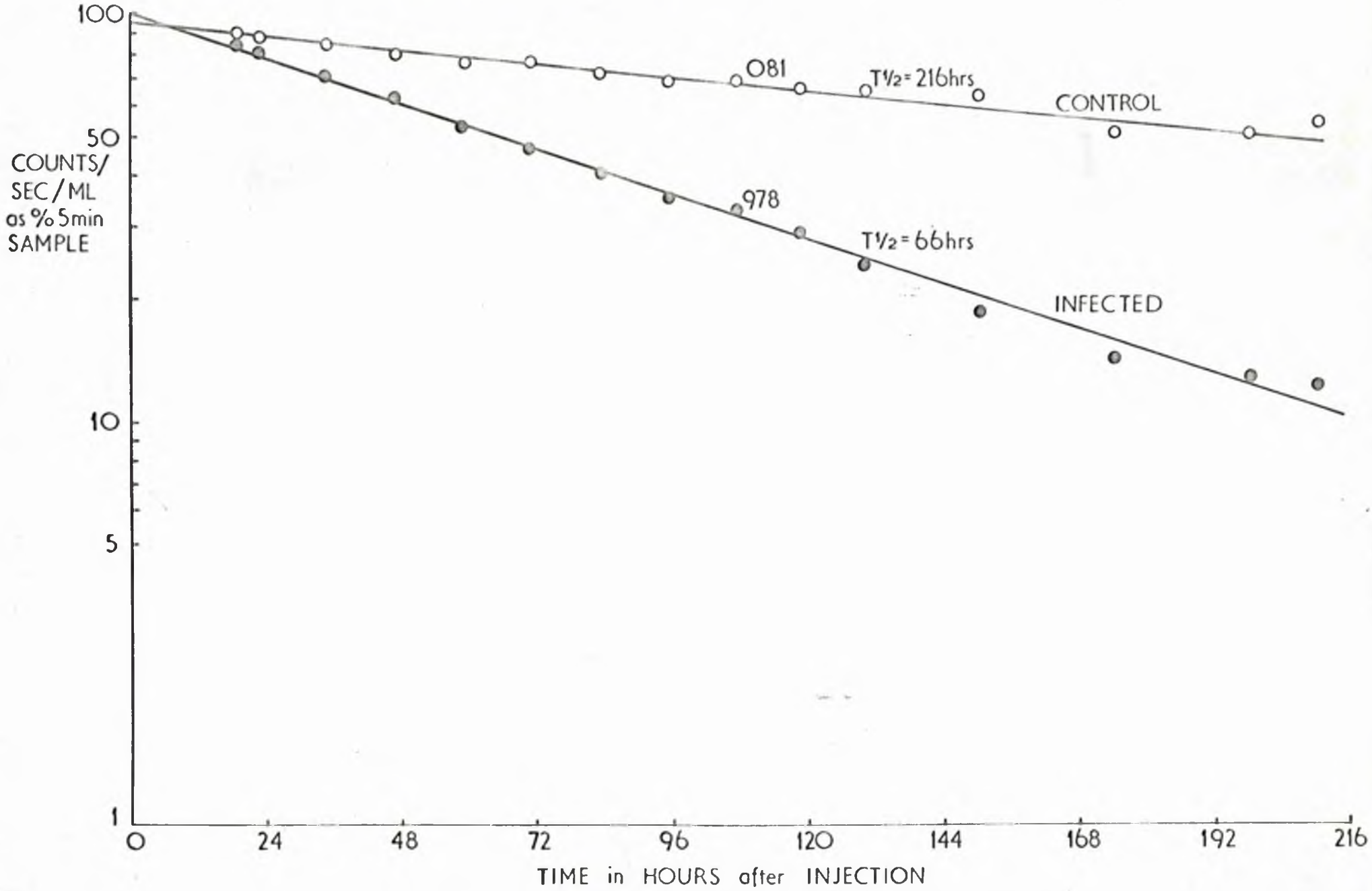
TABLE 16

WHOLE BLOOD AND CIRCULATING RED CELL VOLUMES

	RABBIT NO.	BLOOD VOLUME (ML/KG)	CIRC. R.B.C. VOLUME (ML/KG)
INFECTED	978	50.6	13.3
	008	48.8	17.1
	083	40.3	8.5
	179	57.4	11.3
	Mean	49.3	12.6
	S.D.	7.0	3.6
CONTROL	890	40.4	16.5
	082	31.5	12.2
	081	35.6	13.4
	990	36.6	15.2
	Mean	36.0	14.3
	S.D.	3.7	1.9
	P	0.05	N.S.

FIGURE 10

^{51}Cr LABELLED RBC-RABBIT (RED CELLS)



shown in Table 17. It is noteworthy that the most anaemic rabbit had the shortest half-life value, whereas rabbit number 008 which was considered to be infected had haematocrit and erythrocyte survival values very similar to those of the normal animals.

While these results clearly show an abnormal disappearance of isotope from the circulation of infected animals, they give no indication of how this situation arises. It could be due to increased loss of red cells, increased intravascular breakdown of cells, or increased elution of isotopes from the cells. The true explanation depends on a quantitative study of the rate and route of excretion of the isotope.

(iii) Excretion of ^{51}Cr in Fluke-infected and Normal Rabbits

(a) Urinary Excretion

In order to assess the importance of excessive haemolysis of red cells and elution of isotope from the cells in causing the rapid removal of labelled erythrocytes from the circulation of the infected rabbits, the total radioactivity excreted in the urine of each animal over the 6-day experimental period was expressed as a % of that injected. In the infected animals an average of 14.6% (S.D. 0.5) of the injected activity was excreted in the urine, while the corresponding figure for the controls was 15.4% (S.D. 2.6).

It is clear that whatever the cause of the reduced half-life of the ^{51}Cr-labelled red cells in the infected rabbits, neither increased haemolysis nor elution of isotope from the cells played any significant part.

^{51}Cr Labelled R.B.C. Turnover Studies in Normal and Fluke-infected Rabbits

TABLE 17

SURVIVAL OF LABELLED ERYTHROCYTES

	RABBIT NO.	P.C.V.	$T_{\frac{1}{2}}$ (Hrs) (Whole Blood)	$T_{\frac{1}{2}}$ (Hrs) (Packed R.B.C.)
INFECTED	978	26	57	66
	008	39	168	141
	083	21	66	60
	179	17	41	41
	Mean	26	83	77
	S.D.	10	58	44
CONTROL	890	39	216	282
	082	38	210	240
	081	42	153	216
	990	39	153	194
	Mean	40	183	233
	S.D.	2	35	38
	P	< 0.05	< 0.05	< 0.002

(b) Faecal Excretion

In the previous section it was established that considerable movement of plasma macromolecules occurred into the intestinal tract of fluke-infected rabbits, and it was suggested that this was the likely cause of the rapid turnover of plasma proteins observed in these animals.

Because of the likelihood that the anaemia was associated with loss of red cells into the gut, the total radioactivity in each 24-hour collection of faeces was divided by the activity per ml. of whole blood taken at the beginning of the collection period, and likewise by the activity per ml. of red cells to give a daily faecal "clearance" of whole blood and red cells respectively. These faecal "clearance" figures represent the amounts of blood or red cells which have to appear in the gastro-intestinal tract to account for the radioactivity in the faeces. The mean results obtained (Table 18) show the marked difference between infected and control animals in the faecal output of ^{51}Cr, expressed both as a "clearance" figure and as a cumulative faecal output, and indicate a striking loss of isotope into the digestive tract of the infected rabbits. "Clearance" figures obtained for each day of the experiment are shown in Appendix B.

This table also shows the number of flukes recovered at autopsy and it is clear that a good correlation existed between the number of flukes present within the bile-ducts and the magnitude of the daily blood loss. However, when this was converted to blood loss per fluke

^{51}Cr Labelled R.B.C. Turnover Studies in Normal and Fluke-infected Rabbits

TABLE 18

AVERAGE DAILY FAECAL "CLEARANCE" OF BLOOD AND RED CELLS (ML)

	RABBIT NO.	P.C.V.	FAECAL "CLEARANCE"		CUMULATIVE FAECAL ACT. (% INJ.)	FLUKES RECOVERED	BLOOD LOSS/ FLUKE
			BLOOD	RED CELLS			
INFECTED	978	26	26.0	5.6	45.6	41	0.63
	008	39	3.1	1.0	15.7	4	0.80
	083	21	15.2	3.5	42.5	33	0.47
	179	17	47.4	7.5	46.4	56	0.85
	Mean	26	23.0	4.4	37.6	34	0.69
	S.D.	10	18.9	2.8	14.7	-	0.12
CONTROL	890	39	0.20	0.07	1.2		
	082	38	0.22	0.07	0.9		
	081	42	0.16	0.06	0.7		
	990	39	0.23	0.09	1.0		
	Mean	40	0.21	0.07	1.0		
	S.D.	2	0.03	0.01	0.2		
	P	<0.05	<0.05	<0.05	<0.01		

recovered, the values ranged from 0.46 to 0.85 ml. (mean 0.69 ml.).

(c) Excretion of ^{51}Cr After Intravenous Injection of Lysed Labelled Cells

When ^{51}Cr-labelled red cells are broken down intravascularly, the isotope is excreted more or less quantitatively in the urine. However, some of the ^{51}Cr is excreted via the bile, and the suggestion has been made that the increase in faecal excretion of ^{51}Cr in fluke-infected animals might be due not to loss of red cells into the gut via flukes and bile, but to increased elution of isotope from the cells in infected animals, accompanied by increased bile flow, (Sinclair, 1967). To check this hypothesis three infected and three control rabbits were injected with a preparation of ^{51}Cr-labelled cells which had been lysed by freezing and thawing. Urine and faeces were collected and assayed for radioactivity for a period of 120 hours after injection. The results of this experiment are shown in Table 19.

Although there was some increase in the faecal excretion of isotope in infected as compared to control animals, this represents the total amount of excretion in 5 days and therefore in the main experiments described the contribution to faecal activity in infected animals from this source is negligible.

Conclusions

Comparison of half-life values of ^{51}Cr-labelled red cells in different groups of animals is only valid if it can be assumed that the circulating red cell volume of each animal remains relatively constant from day to day, that red cell production and destruction are in

URINARY AND FAECAL EXCRETION OF ^{51}Cr OVER 120 HOURS

IN NORMAL AND FLUKE - INFECTED RABBITS

AFTER THE INTRAVENOUS INJECTION OF LYSED ^{51}Cr - LABELLED RED CELLS

TABLE 19

	RABBIT NO.	% OF INJECTED DOSE RECOVERED	
		URINE	FAECES
CONTROL	890	55.5	0.2
	990	41.0	0.8
	082	63.3	0.5
	Mean	53.3	0.5
	S.D.	11.3	0.3
INFECTED	979	33.2	9.2
	083	49.9	5.2
	084	64.8	7.5
	Mean	49.3	7.3
	S.D.	15.8	2.0
	P	N.S.	< 0.01

equilibrium, and that the rate of elution of the isotope from the cells is the same for both groups.

Since the red cell volumes of both groups of animals were more or less maintained throughout the course of the experiment, it may be concluded that the rapid fall in red cell activity exhibited by the infected group was not caused by an increase in circulating red cell mass. However the rate of decline of radioactivity not only reflects the rate at which the labelled cells are lost from the circulation but also the rate at which newly produced unlabelled cells "dilute" those labelled cells already present. The fact that the red cell mass of the infected animals did not decrease during the experiment, indicates that the rapid loss of erythrocytes from the circulation was compensated for by an equivalent increase in red cell production.

The finding of markedly different values for whole blood and red cell half-life in the control rabbits (Table 17) illustrates the different responses of the two groups of animals to the removal of 14 ml. of blood over a period of 8 days for radioactivity determinations. In the normal rabbits both P.C.V. and red cell volume fell very slightly during the experiment. As a result, fewer red cells (and hence less radioactivity) was present per ml. of whole blood as the study proceeded. In this group the rate of replacement of labelled with unlabelled cells could not quite have kept pace with the rate of removal of red cells for blood sampling resulting in the non-parallel decline in red cell and whole blood radioactivity. In the infected animals however, removal

of blood for radioactivity determinations did not reduce either the P.C.V. or red cell volume. The parallel decline in red cell and whole blood activity indicates that the originally injected labelled erythrocytes were being "diluted" with unlabelled cells at a very much faster rate, confirming that, in this group of rabbits, erythropoiesis must have been greatly increased.

In the experiment described there was no difference in urinary excretion of isotope between fluke-infected and control animals, indicating that fascioliasis neither increases intravascular breakdown nor leads to the production of erythrocytes from which chromium is eluted at a more rapid rate than normal.

It would appear from the results shown in Table 18 that the main cause of the rapid loss of isotope from the circulation of the infected rabbits was the passage of red cells into the gastrointestinal tract, presumably via the bile. However, it is not possible to regard the clearance figures shown in Table 18 as giving absolute values for blood loss, since some small contribution may arise due to the passage of ^{51}Cr into the gut via the bile (Table 19). Since it is likely that much of the ^{51}Cr from the haemolysed red cells was present in the plasma following injection, much of the increased removal of isotope via the bile in infected rabbits could probably be accounted for by increased movement of plasma proteins into the gut of these animals as a result of the feeding activities of the flukes. This, coupled perhaps with an increased bile flow (caused by mechanical stimulation of bile duct smooth muscle) may have resulted in the increased removal of chromium by this route.

A second possible source of error in the "clearance" figures obtained may have resulted from the assumption that the haematocrit of the blood in the hepatic capillaries was the same as that of peripheral venous blood. If, for example the blood vessels around the bile ducts contain blood of a lower haematocrit than that represented by a venous blood sample, the "clearance" shown in Table 18 will underestimate the amount of blood actually removed. However it seems unlikely that large differences in haematocrit exist between peripheral venous and liver haematocrit (Gibson, Seligman, Peacock, Aub, Fine and Evans 1946), and it is therefore reasonable to assume that the quoted figures for blood and red cell "clearances" are very close to the actual amount of blood lost.

A good correlation was obtained between the degree of anaemia observed (as indicated by venous haematocrit), the magnitude of the blood loss suffered by each of the infected rabbits, and the number of flukes recovered at autopsy. Thus, the most anaemic animal (rabbit number 179, with a P.C.V. of 17%) appeared to lose daily about 30% of its circulating red cell volume into the gastro-intestinal tract. This rabbit was also carrying the greatest burden of parasites (56 flukes). On the other hand, rabbit number 003 (from which only 4 flukes were recovered) lost about 1 ml. of red cells per day into the gut. Although this animal outwardly appeared normal (P.C.V. 39%) it was still losing about 15 times more blood than any of the control rabbits, demonstrating the sensitivity of this technique for the detection of enteric blood loss. The daily loss of red cells in each of the other infected rabbits

was equivalent to about 14% of their red cell volumes.

Bearing in mind that the circulating red cell volume of these animals did not fall over the experimental period, it is clear that blood loss of this magnitude must have been compensated for by a substantial increase in red cell production.

(B) IRON METABOLISM IN NORMAL AND FLUKE-INFECTED RABBITS

A more quantitative and direct measurement of erythropoiesis was obtained by following the plasma clearance of an intravenously injected dose of ^{59}Fe. This, together with the serum iron level at the time of injection enabled calculation of the absolute plasma iron turnover rate. It is assumed that this provides a reasonable estimate of the rate at which iron was utilised for red cell production. In order to carry out such a study, it is also necessary to assume that the injected radioiron does not change the steady state of the animals with respect to iron metabolism. For this purpose ^{59}Fe of high specific activity was obtained from the Radiochemical Centre, Amersham, as a sterile solution of ^{59} ferric citrate. Since only 6 µg of iron was injected into each animal it was considered that the validity of this assumption was upheld.

(i) Ferrokinetic Studies

Six fluke-infected (50 metacercariae each 3 months previously) and two normal rabbits were established in metabolism cages and their plasma volumes measured. Approximately 24 hours later each was injected with 50 µc of ^{59}Fe as ferric citrate and heparinised blood samples

collected at 5, 10, 20, 30, 45, 60, 90, 120, 180 and 360 minutes after injection. Further samples were taken daily for 14 days and thereafter on alternative days for a further seven days. Complete 24-hour collections of urine and faeces were made throughout the experiment.

Venous haematocrit and blood haemoglobin measurements were carried out daily, red cell counts at the beginning and end of the experiment, and serum iron determinations on samples obtained immediately prior to injection of radioiron. With the figures obtained, the mean corpuscular volume (MCV) and mean corpuscular haemoglobin volume (MCHC) were estimated for each rabbit.

$$MCV = \frac{P.C.V. \times 10}{R.B.C. \times 10^6 \text{ cu.mm.}}$$

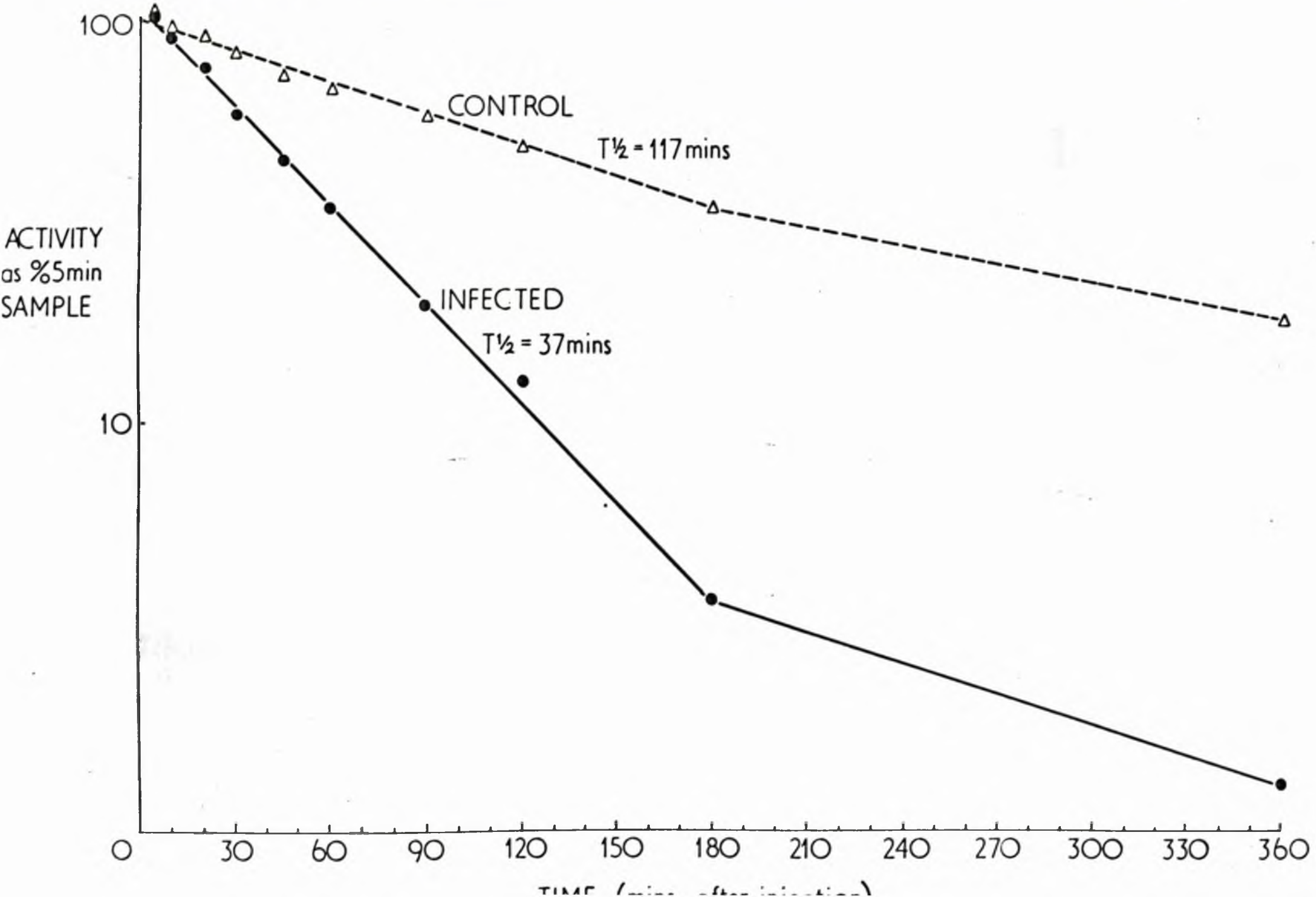
$$MCHC(\%) = \frac{\text{Haemoglobin (gm\%)} \times 100}{P.C.V.}$$

The haematological data on the two groups of animals are shown in Table 20. These show the anaemia to be of the macrocytic hypochromic type, indicating some disturbance of the iron metabolism. However, no significant difference in serum iron levels (Table 21) was apparent between the two groups of animals.

The radioactivity of each plasma sample was expressed as a percentage of the 5 minute post-injection value, and a semi-log plot made of the activity against time. Mean curves obtained for normal and fluke-infected animals (Fig. 11) show the significantly more rapid clearance rate of Fe from the plasma of the infected animals. This rate is expressed quantitatively as a half-life ($T\frac{1}{2}$) value in Table 21, calculated from the plasma activities obtained during a 3-hour period

FIGURE 11

MEAN PLASMA ^{59}Fe DISAPPEARANCE CURVES in NORMAL and FLUKE-INFECTED RABBITS



^{59}Fe Labelled R.B.C. Turnover Studies in Normal and Fluke-infected Rabbits

TABLE 20

ERYTHROCYTE STUDIES

	RABBIT NO.	P.C.V.	Hb (gm/100ml)	R.B.C. (10^6 cu.mm)	MCV (cu.μ)	MCHC (%)
INFECTED	306	23	5.7	2.54	90.6	24.8
	416	40	12.5	5.05	79.2	31.3
	315	26	7.5	3.00	86.7	28.8
	363	28	7.7	3.15	88.9	27.5
	177	30	5.6	3.43	87.0	18.7
	230	35	10.8	3.92	89.3	30.9
	Mean	30	8.3	3.51	87.0	27.0
	S.D.	6	2.8	0.93	4.1	4.7
CONTROL	377	40	14.6	5.63	70.4	37.0
	361	42	14.9	5.63	72.2	36.3
	Mean	41	14.8	5.63	72.2	36.3
	S.D.	1	0.1	-	2.5	1.1
	P	<0.05	<0.02	<0.02	<0.01	<0.05

PLASMA ^{59}Fe TURNOVER STUDIES IN NORMAL AND FLUKE-INFECTED RABBITS

TABLE 21

	Rabbit No.	Plasma Volume (ml/kg)	Serum Iron (µg/100ml)	Half-life (mins)	Plasma Iron Turnover (mg/day/ 100ml blood)	Total Turnover (mg/day)	Flukes Recovered
INFECTED	306	41.3	110	21	3.93	3.68	27
	416	26.0	125	53	1.42	1.96	7
	315	40.0	138	42	2.43	2.50	22
	363	43.0	147	35	2.94	3.46	28
	177	35.6	121	27	3.23	3.53	25
	280	27.7	147	46	2.08	2.25	16
	Mean	35.6	131	37	2.67	2.90	21
	S.D.	7.2	15.1	12	0.89	0.75	
CONTROL	377	27.8	128	153	0.54	0.84	
	361	26.3	92	81	0.63	0.58	
	Mean	27.1	115	117	0.59	0.71	
	S.D.	1.1	32.5	51	0.06	0.18	
	P	N.S.	N.S.	< 0.01	< 0.02	< 0.01	

following injection. Since the concentration of the tracer as a function of time declined exponentially (at least over the first 3 hours following injection) the plasma iron turnover rate could be calculated in accordance with first-order kinetics.

$$\text{i.e.} \quad k = \frac{0.693}{T_{\frac{1}{2}} \text{ (mins.)}}$$

where k represents the fraction of iron in the plasma turned over per unit of time. Quantitative estimates of plasma iron turnover were obtained from the radioiron disappearance data and the total serum iron using the formula of Bothwell *et al* (1957).

$$\text{Plasma Iron Turnover (mg./day/100ml. blood)} = \frac{\text{Serum Iron (mg./100ml.)} \times 0.693 \times 1440}{T_{\frac{1}{2}} \text{ (mins.)}} \times \frac{100 - \text{Hct}}{100}$$

The total plasma iron turnover for each animal was then calculated from this figure and the blood volume (Table 21).

In these studies, it is assumed that plasma iron turnover reflects iron utilised inhaemoglobin or red cell synthesis and that other tissues have insignificant turnover. It is clear from Figure 11 that plasma iron disappearance curves cannot be fully represented as single exponential functions. They are, in fact, complex curves reflecting both the departure of radioiron and its constant re-entry into the plasma from the tissues. The initial more rapid phase (from which the plasma iron turnover rate was calculated) may be assumed to represent almost entirely the rate at which plasma radioiron is removed by the bone marrow for red cell production. Although this is not strictly true

because of the contribution of other processes such as equilibration within the labile iron pool and transfer to cytochromes and catalases, the small amount of iron involved in such processes, renders their contribution to the overall plasma iron turnover rate insignificant. Even in the normal rabbit, 90% of the iron leaving the plasma at any time is directed towards the marrow for haemoglobin synthesis (Noyes, Hosain and Finch 1964).

Another possible source of error in estimating erythropoiesis from plasma iron turnover measurements is early recycling of ^{59}Fe from rapidly destroyed erythrocytes, but again, this is unlikely to materially alter the results since comparatively few labelled red cells are present in the peripheral circulation during the 3 hour post-injection period.

The slower decline in plasma activity commencing about 3 hours post-injection was probably the result of feed-back into the plasma of the small amount of injected radioiron which was directed to other parts of the labile iron pool such as the liver and spleen following injection. It is clear that in the infected animals, since more than 95% of the radioactivity was cleared exponentially within 3 hours, the contribution of these tissues to the plasma iron turnover rate was negligible. In the normal animals however, the values obtained will tend to be more of an overestimate of the rate of iron utilisation, since only about 70% of the initially injected dose was cleared within the 3-hour post-injection period, and return of radioiron from the tissues would make a more significant contribution to plasma radioactivity.

The validity of the plasma iron turnover rate as a measure of erythropoiesis in these rabbits may be checked by calculating the amount of iron theoretically required for red cell production. If an erythrocyte life-span of 50 days (as estimated by Brown & Eadie, 1953) is assumed, the amount of iron required for haemoglobin synthesis in the control rabbits used in this experiment would have been about 0.83 mg/day. Thus, the figure of 0.71 mg/day may be used with confidence as an estimate of the amount of iron normally required for red cell production in the rabbit.

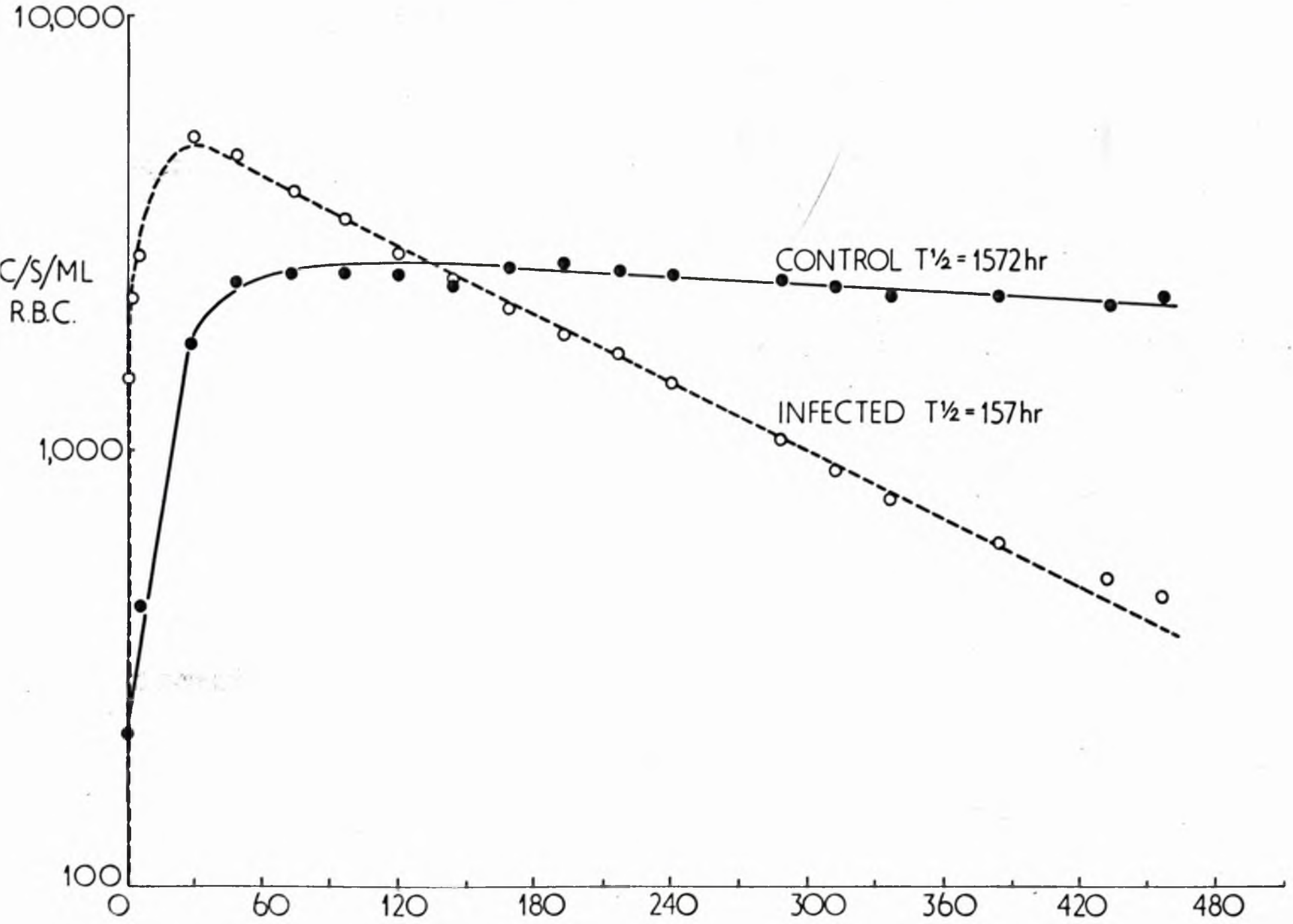
It is apparent from the results shown in Table 21 that erythropoiesis as measured by plasma iron turnover was proceeding at a significantly faster rate in fluke-infected than normal rabbits. This was confirmed by following the rate of incorporation of the radioiron into circulating red cells.

A semi-log plot of the activity per ml. of red cells against time calculated from the radioactivity and haematocrit of each blood sample was made. Mean curves obtained for infected and normal rabbits are shown (Fig. 12). The initial sharp rise in red cell activity was much more apparent in the infected animals due to the mean utilisation of 86% of the injected dose for red cell production as compared to only 48% in the controls. Thereafter a significantly more rapid decline in the erythrocyte activity of the infected rabbits occurred (Fig. 12). This was expressed as a half-life value (Appendix B). The mean half-life of the ^{59}Fe-labelled erythrocytes in the infected rabbits was

FIGURE 12

TURNOVER of ^{59}Fe LABELLED RBC's in NORMAL and FLUKE-INFECTED RABBITS

MEAN ^{59}Fe LABELLED RBC. DISAPPEARANCE CURVES



157 hours (S.D. 123) while the corresponding figure for the controls was 1572 hours (S.D. 371).

In previous kinetic studies on man using radioiron, attempts were made to calculate the rate of red cell formation using the plasma iron turnover in conjunction with the maximum percentage of the injected radioactivity subsequently appearing in the red cells. This was not done in the present experiment because it was impossible to know accurately what point on the red cell utilisation curve represents that part of the radioiron which proceeds directly to the marrow as compared to the iron originally taken up by other tissues and later re-routed to the marrow.

(ii) Faecal Excretion of ^{59}Fe in Fluke-Infected and Normal Rabbits

The explanation of the abnormal disappearance of isotope from the circulation of infected animals was apparent from a quantitative study of the radioactivity excreted. This is expressed in Table 22 as a "faecal clearance" of whole blood and of red cells, obtained by dividing the total activity of each 24-hour collection of faeces by the activity of whole blood and red cells respectively taken at the beginning of the collection period. (Daily "clearances" for each rabbit are shown in Appendix B). There was a very striking difference between the figures for the normal and the fluke-infected rabbits, and the magnitude of the blood loss correlates well with the number of flukes recovered at autopsy.

From the haemoglobin concentration in the blood at the beginning of each collection period the loss of haemoglobin iron was calculated,

TABLE 22

AVERAGE DAILY LOSS OF WHOLE BLOOD, RED CELLS AND HAEMOGLOBIN IRON

	Rabbit No.	Whole Blood (ml)	R.B.C. (ml)	Haemoglobin Iron Excreted in Faeces (mg/day)	Flukes Recovered
INFECTED	306	16.50	3.57	3.10	27
	416	4.46	1.64	1.75	7
	315	10.41	2.59	2.52	22
	363	14.04	3.72	3.50	28
	177	12.02	3.42	3.05	25
	280	7.90	2.73	2.47	16
	Mean	10.88	2.93	2.73	21
	S.D.	4.31	0.79	0.62	
CONTROL	377	0.94	0.38	0.45	
	361	0.32	0.12	0.14	
	Mean	0.63	0.25	0.30	
	S.D.	0.44	0.18	0.22	
	P	< 0.02	< 0.01	< 0.002	

each gram of haemoglobin containing 3.34 mg. iron. The markedly increased loss of iron in the faeces of the infected compared to the control group is similarly shown in Table 22, and again the magnitude of the loss correlates fairly well with the number of flukes present.

It would also appear that the increased plasma iron turnover rate was quantitatively associated with the marked faecal loss of iron in the infected animals. Thus the mean daily plasma iron turnover rate of the infected rabbits was 2.90 mg.(S.D.O. 75), while the average daily faecal loss of iron was 2.73 mg. (S.D.O.62). In addition, a good correlation existed in individual animals between the magnitude of the iron loss and the corresponding plasma iron turnover rate (Tables 21 and 22).

While these results clearly show that rabbits infected with F. hepatica lose significant amounts of iron in the faeces, they give no indication of the ability of these animals to re-absorb iron lost into the gastrointestinal tract. This was studied in two further experiments.

In the first of these, the red cells of groups of normal and infected rabbits were doubly labelled with ^{59}Fe and ^{51}Cr. Faecal "clearance" measurements of the two isotopes enabled a calculation to be made of the haemoglobin iron reabsorbed from the gut. The second experiment involved the faecal recovery of ^{59}Fe and ^{51}Cr after the administration of oral doses of lysed doubly labelled red cells.

(iii) Studies with Red Cells Labelled with ^{59}Fe and also with ^{51}Cr

(a) Fate of Intravenously Injected Labelled Cells

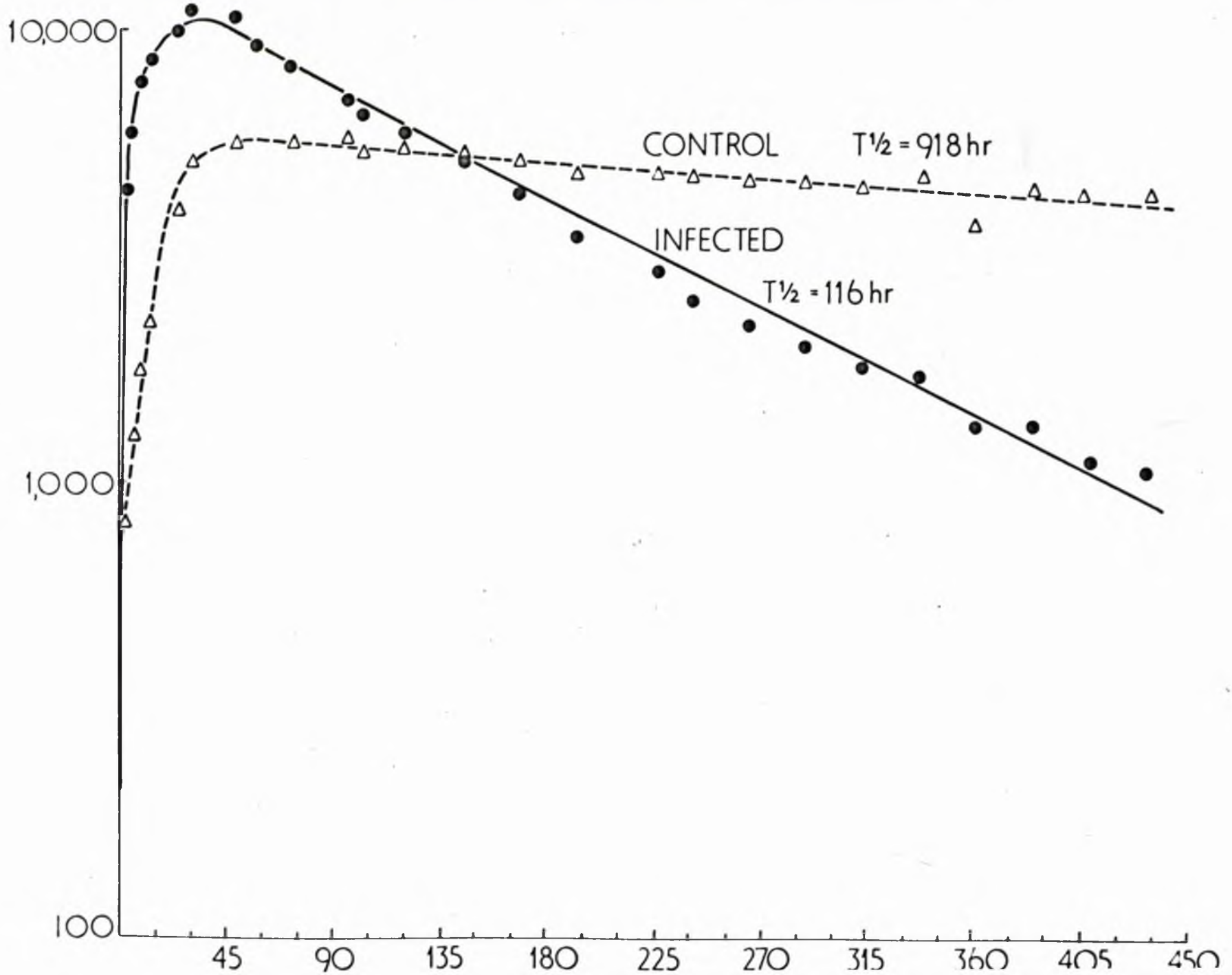
Six fluke-infected (50 metacercariae 3 months previously) and 4 normal rabbits were each injected intravenously with 30 μc ^{59}Fe (specific activity 14 μc/μg) as ^{59} ferric citrate, and haparinised blood samples taken daily for 21 days. Five days following labelling with ^{59}Fe, samples of blood were withdrawn from each rabbit, the red cells labelled with ^{51}Cr and re-injected. Blood samples collected at appropriate times were assayed for radioactivity.

Red cell counts were carried out at the beginning and at the end of the experiment, while venous haematocrit and blood haemoglobin were determined daily. Haematological indices were calculated and the results shown in Appendix B. The infected group all showed marked reduction in P.C.V. haemoglobin concentration, and red cell count ($P = < 0.001$) while the values obtained for MCV and MCHC confirmed the previous findings of hypochromic macrocytic type of anaemia.

The ^{59}Fe activity of each blood sample expressed as activity per ml. of red cells was plotted on a semi-log scale against time. The mean curves obtained for the normal and infected rabbits are shown in Figure 13. A semi-log plot of ^{51}Cr activity per ml. of red cells against time was similarly made, in this case activity being expressed as a percentage of the 10-minute post-injection blood sample. Mean curves for normal and infected rabbits are shown in Fig. 14. The significantly shortened half-lives of both ^{59}Fe and ^{51}Cr-labelled

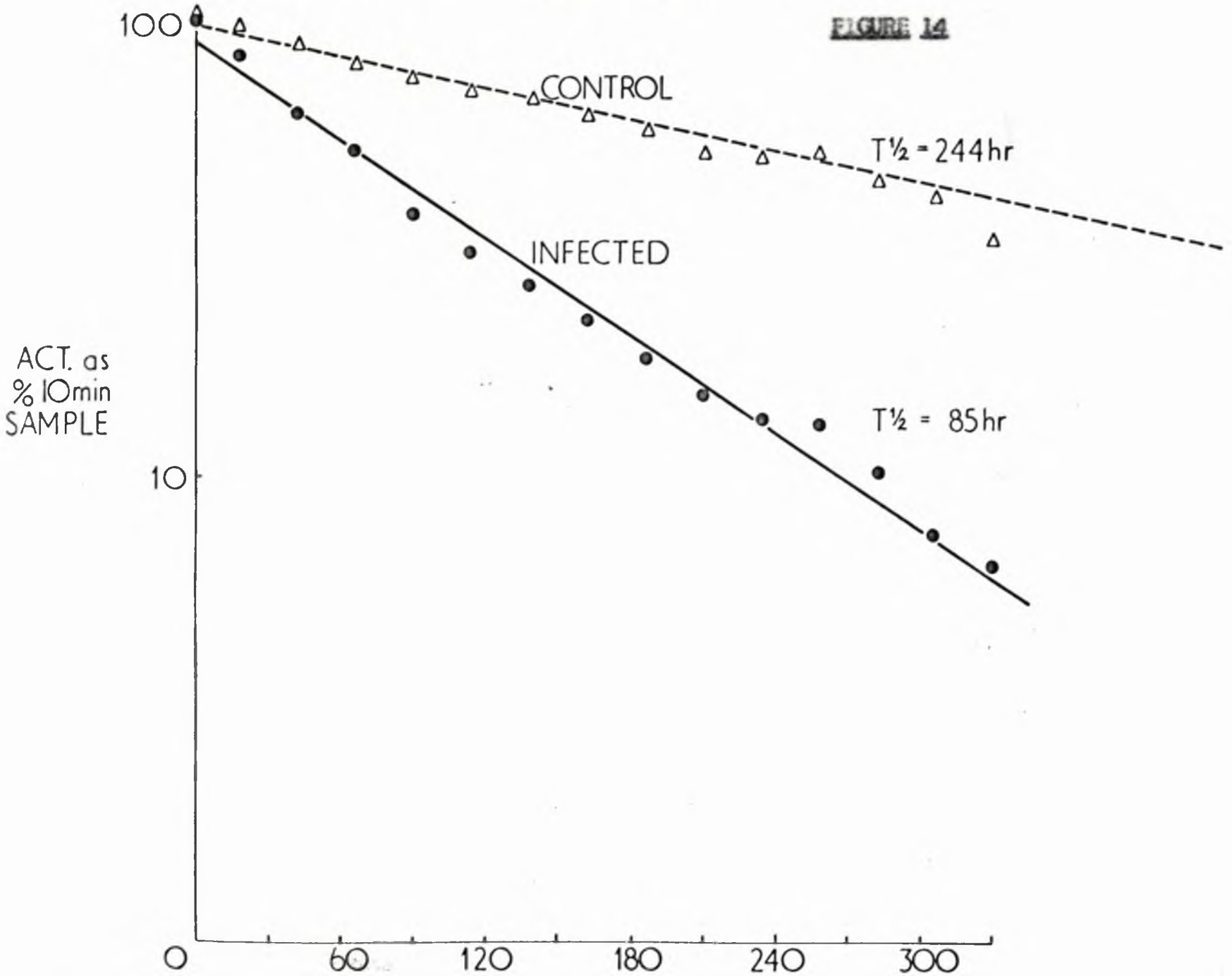
FIGURE 13

TURNOVER of ^{59}Fe and ^{51}Cr LABELLED R.B.C's in NORMAL and FLUKE INFECTED RABBITS. MEAN ^{59}Fe LABELLED R.B.C. DISAPPEARANCE CURVES.



TURNOVER of ^{59}Fe and ^{51}Cr LABELLED RBC's in NORMAL AND FLUKE INFECTED RABBITS MEAN ^{51}Cr LABELLED R.B.C. DISAPPEARANCE CURVE

FIGURE 14



erythrocytes in the infected animals relative to the controls is apparent. The individual values are shown in Appendix B.

A shorter half-life of ^{51}Cr-labelled cells relative to those labelled with ^{59}Fe was evident in the control group ($P < 0.002$) while in the infected animals, this difference was insignificant ($P > 0.1$). This indicates that ^{59}Fe lost from the circulation was not re-utilised for further haemoglobin synthesis, and was instead excreted, whereas in the control group, this iron was retained and transported by means of the iron-binding plasma protein to the marrow where it was incorporated into new erythrocytes. Thus the main site of destruction of the red cells in the two groups was fundamentally different.

The amount of iron lost into the gut was estimated each day following injection of the ^{51}Cr-labelled red cells by the method of Roche et al (1957)

$$\text{mg. iron lost into intestine} = \frac{\text{Haemoglobin (gm./100 ml.)} \times 3.34 \times {}^{51}\text{Cr blood "clearance" (ml.)}}{100}$$

The same formula was used in determining iron lost in the faeces, measured by ^{59}Fe faecal activity. From Table 23, it is apparent that there was an insignificant difference between the amount of iron lost into the gut and that excreted in the faeces of the infected animals. Daily faecal "clearances" together with the amounts of iron lost into the gut and excreted in the faeces of each animal are shown in Appendix B.

It is of interest to note that in all cases, and especially in the control animals, the average amount of iron lost into the gut was

^{59}Fe / ^{51}Cr-labelled R.B.C. Turnover Studies in Normal and Infected Rabbits

TABLE 23

AVERAGE DAILY LOSS OF WHOLE BLOOD, RED CELLS AND HAEMOGLOBIN IRON

	Rabbit	Average Daily ^{59}Fe Clearance(ml)		Average Daily ^{51}Cr Clearance(ml)		Haemoglobin Iron(mg/day)		Flukes Recovered
		Whole Blood	R.B.C.	Whole Blood	R.B.C.	Lost into Gut	Excreted in Faeces	
INFECTED	339	4.9	1.5	4.8	1.5	1.5	1.6	10
	335	12.5	2.6	13.0	2.8	0.9	1.0	27
	340	11.2	2.5	11.5	2.6	3.0	3.2	22
	316	14.0	3.0	15.8	3.4	3.2	2.9	23
	BL	15.2	3.1	14.4	3.1	2.6	2.9	27
	334	10.9	3.1	10.9	3.1	3.3	3.4	21
	Mean	11.4	2.6	11.7	2.8	2.4	2.5	22
	S.D.	3.6	0.6	3.9	0.7	0.9	0.9	
CONTROL	344	0.4	0.2	0.1	0.04	0.04	0.13	
	179	0.5	0.2	0.1	0.04	0.05	0.16	
	342	0.8	0.2	0.1	0.04	0.04	0.18	
	201	0.5	0.2	0.1	0.03	0.03	0.15	
	Mean	0.5	0.2	0.1	0.04	0.04	0.16	
	S.D.	0.2	-	-	-	-	0.02	
	P	<0.001	<0.001	<0.001	<0.001	<0.002	<0.001	

less than that excreted in the faeces. The reason for this apparent anomaly is that faecal iron is derived not only from senescent red cells via the bile, but also from desquamation of intestinal iron-containing cells. Thus, calculation of the amount of iron lost into the gut from ^{51}Cr faecal activity results in underestimation of the normal iron loss. However, it is apparent that since loss by sloughing of intestinal cells forms such a small part of the total, it may be ignored for the purpose of measuring the faecal iron loss suffered by the infected rabbits.

(b) Fate of Orally Administered Labelled Cells

The rabbits' ability to reabsorb haemoglobin iron from the gut was also estimated by administering an oral dose of lysed red cells which had been labelled with ^{51}Cr and ^{59}Fe to a group of infected animals and subsequently following the blood and faecal radioactivities.

No radioactivity was detected in the blood of these rabbits during the 5-day period following the oral dose. Absorption was thus calculated as the difference between the total radioactivity administered and that which accumulated in the faeces. The total excreted activity of ^{59}Fe and ^{51}Cr as a % of the injected activities are shown in Table 24.

Since on average 95% (S.D. 8) labelled haemoglobin had appeared in the faeces by 5 days following oral administration it seems reasonable to assume that no significant absorption of ^{59}Fe occurred following the dose.

Oral ^{59}Fe / ^{51}Cr-labelled R.B.C. Absorption Experiment

TABLE 24

EXCRETED ACTIVITIES OF ^{59}Fe and ^{51}Cr

Rabbit No.	Total ^{51}Cr Excreted Activity as % Inj.	Total ^{59}Fe Excreted Activity as % Inj.
306	75.2	79.1
416	95.7	98.8
315	98.2	99.0
363	91.6	94.6
177	86.1	99.8
280	86.0	97.8
Mean	88.8	94.8
S.D.	8.3	7.9

Conclusions

The anaemia associated with F. hepatica infections of rabbits is characterised by macrocytosis and hypochromia indicating that some disturbance in iron metabolism is associated with the disease. While the finding of a rapid clearance of radioiron from the plasma suggests that the bone marrow activity of the infected rabbits was greatly increased, it was nevertheless necessary to measure the serum iron level and the rate of appearance of ^{59}Fe into newly formed erythrocytes in order to eliminate the possibility that marrow hyperplasia was associated either with ineffective erythropoiesis as occurs in anaemia caused by maturation failure, or a deficiency of iron.

The greater rapidity with which the radioiron was utilised for red cell production in the infected rabbits relative to the controls is apparent from Figs. 12 and 13. In the former group, the maximum ^{59}Fe uptake by the red cells was found within 24 hours of injection, but this was not attained until 8 days post-injection in the controls, showing that the iron was in fact used for effective erythropoiesis, and was not taken up by an "inactive" hyperplastic marrow. Furthermore anaemias of dyshaemopoietic aetiology, in addition to rapid ^{59}Fe clearances, are usually characterised by abnormally high levels of serum iron. Since no increase in serum iron was found to be associated with the infected rabbits, it may be concluded that diminished erythropoiesis was not responsible for the anaemia.

The normality of the serum iron levels of the infected rabbits, despite the rapidity with which this element was removed from the

circulation, also excludes the possibility of iron deficiency being the primary cause of the anaemia. This finding is consistent with the absence of microcytes in the peripheral blood of these animals. However, although the plasma iron turnover rates (Table 21) revealed that fluke-infected rabbits used about four times as much iron each day for haemoglobin synthesis, it seems likely in view of the hypochromia associated with the anaemia that even this large amount of iron was not sufficient to satisfy the requirements of the marrow.

The more rapid removal of ^{59}Fe from the plasma of the infected rabbits, together with the striking increase in the rate at which the labelled erythrocytes subsequently disappeared from the circulation (Figs. 11 and 12) can only be interpreted as indicating that the bone marrow of these animals was stimulated in response to an increased loss of red cells. While this loss could in theory have resulted from excessive haemolysis, it is clear from Tables 22 and 23, that it was in fact associated with the passage of considerable amounts of red cells (and hence iron) into the gastrointestinal tract of the infected rabbits. This loss was an average equivalent to 10% of the circulating red cell volume of the 12 infected rabbits studied.

The good correlation obtained between the plasma iron turnover rate (Table 21) and the magnitude of the faecal iron loss suffered by the infected rabbits (Table 22) provides further evidence of the validity of this technique as a measure of erythropoiesis.

It is noteworthy that the iron derived from the red cells which

passed into the gut was not significantly reabsorbed (Table 23). This result was confirmed by the quantitative excretion of an oral dose of ^{59}Fe / ^{51}Cr-labelled red cells (Table 24) and it would therefore appear that even the anaemic rabbit was unable to absorb other than dietary inorganic iron.

DISCUSSION

The results of the experiments described in this section of the thesis go far in explaining the cause of the macrocytic hypochromic anaemia observed in rabbits infected with F. hepatica, and throughout these studies, several features consistently distinguished the fluke-infected from normal rabbits.

It has been suggested that dyshaemopoiesis, due for example to vitamin B_{12} or protein deficiency may be responsible for the anaemia associated with chronic fascioliasis (Sinclair 1964). Although several authors have reported that F. gigantica may preferentially absorb vitamin B_{12} (Obara et al. 1964; Sewell 1965), the more rapid uptake of injected ^{59}Fe by new erythrocytes, and the normal serum iron levels found in the infected rabbits suggest that red cell production in these animals, far from being depressed, was in fact proceeding at a rate roughly four times that of the controls (Table 21). Thus the theory that absorption of vitamin B_{12} leads to an impairment of erythropoiesis may be removed from consideration as the cause of the anaemia. A lack of essential amino acids is also unlikely to cause the anaemia since amounts of these substances sufficient for erythropoiesis become available from some body source even when the serum protein levels and the protein intake are exceedingly low (Castle 1954).

Further evidence of increased red cell production being associated with F. hepatica infections has also been obtained from comparison of the bone marrow of normal and infected rabbits and sheep (Urquhart 1955;

Sinclair 1964; Sewell *et al* 1968). These authors noted that the amount and activity of the erythroid marrow was in all cases considerably greater in the infected animals. The above results together with the presence of reticulocytes in the peripheral blood of fluke-infected rabbits and sheep (Urquhart 1955; Jennings and Armour 1969) support the view that instead of diminished production of red cells, an increased loss was the primary influence in causing the anaemia associated with the disease.

The more rapid removal of both ^{51}Cr and ^{59}Fe-labelled erythrocytes from the circulation which was so clearly demonstrable in all the infected rabbits is consistent with this theory. While the loss of labelled cells could in theory have resulted from increased intravascular breakdown, it is clear that since the urinary excretion of isotope was almost identical in both normal and infected animals, excessive haemolysis played no part in its aetiology. This result is supported by the normal plasma bilirubin levels found in fluke-infected rabbits, rats and calves (Urquhart 1955; Thorpe 1965; Ross *et al*. 1966). Urquhart (1955) also noted that the spleens of infected rabbits were normal in size and showed no evidence of erythrophagocytosis when compared to those of normal rabbits. For example, the Prussian blue reaction for stainable iron, which is normally positive in haemolytic conditions, was almost entirely absent. Thus the theory that *F. hepatica* secretes a haemolytic toxin may also be excluded from serious consideration as a contributory factor in the aetiology of the anaemia suffered by fluke-infected animals.

The striking faecal loss of isotope associated with all the infected rabbits used in the above studies lends further support to the theory first put forward by Jennings et al (1956) that the primary cause of the anaemia of F. hepatica infections is haemorrhage into the gastro-intestinal tract presumably via the bile. In the ^{51}Cr experiment, an average daily blood loss of 23 ml. occurred into the gut in fluke-infected rabbits, although this was to a large extent dependent upon the number of flukes present within the bile ducts (Table 18). Making the assumption that this loss was caused almost entirely by the blood-sucking activities of the parasite, the average amount of blood consumed per fluke was 0.69 ml. per day. Because of the much smaller fluke burden in the experiments using ^{59}Fe-labelled red cells, the average daily blood loss was 11 ml. (Tables 22 and 23). However when calculated on the basis of blood loss per fluke, the average figure obtained was 0.54 ml. per day, a value very close to that obtained with the higher parasite burden. Since a daily blood loss of 5 ml. over several months is sufficient to produce a marked anaemia in rabbits (Steele 1933; Oberg 1949; Urquhart 1955) it would seem reasonable to suggest that the magnitude of the blood loss suffered by the infected rabbits, albeit into the gut, was sufficient to cause the anaemia associated with the disease. Holmes (1969) has shown that a considerable loss of blood (equivalent to about 0.5 ml. per fluke/day) is similarly associated with F. hepatica infections in sheep.

In an effort to artificially induce anaemia of the type found in F. hepatica infection, several authors have subjected groups of animals

to daily removal of blood and compared the resulting anaemia to that of corresponding groups of fluke-infected animals.

While it is generally recognised in man that the anaemia resulting from chronic blood loss is usually of the normocytic normochromic type, it is also well known that this may subsequently develop into a microcytic hypochromic anaemia because of an associated deficiency of iron. However, it is now becoming increasingly apparent that the type of anaemia resulting from chronic blood loss in domestic animals depends on the volume and duration of this loss. Thus Urquhart (1955) found that daily removal of about 5% of the blood volume from normal rabbits was sufficient to produce a macrocytic hypochromic anaemia beginning at about 6 weeks after bleeding had commenced. This author further showed that this post-haemorrhagic anaemia, in common with that associated with chronic fasciolíasis in this species, was accompanied by marked reticulocytosis and concluded that the anaemias were essentially identical.

Sinclair (1964) compared the anaemia of groups of sheep infected with 600 metacercariae to that of a group subjected to daily removal of 60 ml. of blood, (i.e. about 2% of the blood volume). This figure was derived from the assumption that about 200 flukes, each removing 0.3 ml. of blood daily would be recovered from the bile ducts. A normocytic normochromic anaemia was noted in both groups but that associated with the infected animals was more severe. In view of the recent work of Holmes (1969) it would seem that comparison of the anaemias of these groups was not valid, and it would perhaps have been more appropriate

to have compared the anaemia of the infected animals to that of a group subjected to daily removal of about 120 ml. of blood.

In a later study Sinclair (1965) compared the anaemia of groups of infected (900 metacercariae) and bled (120 ml./day) sheep. In the bled sheep, macrocytosis was noted during the first six weeks but after about 18 weeks of daily bleeding this anaemia developed into a microcytic, hypochromic (iron deficiency) type. No significant change however was apparent in the cell indices of the infected sheep, although slight macrocytosis was found. Unfortunately, since less than 100 flukes were recovered from the infected sheep, comparisons of the two groups was again inappropriate, although as expected, in many ways the bled sheep showed the more severe anaemia. This was demonstrated by the more rapid clearance of an intravenously injected dose of ^{59}Fe from the plasma and subsequent incorporation into new erythrocytes, and the more marked depletion of marrow haemosiderin.

Todd and Ross (1968) also produced a macrocytic hypochromic anaemia in sheep following daily removal of 300 ml. of blood.

The above findings are all the more interesting in view of the recent results of Jennings and Armour (1969). These authors also noted that the anaemia found in the fluke-infected sheep was initially normocytic normochromic, but have further shown that as the disease progressed, reticulocytosis, macrocytosis and hypochromia became more prominent features.

It is thus most likely that the anaemia of fascioliasis in sheep

in common with that produced by haemorrhage varies with the fluke-burden and the duration of the infection.

The macrocytosis which characterised the anaemia resulting from chronic fascioliasis and blood letting in rabbits and sheep is in marked contrast to the normocytic anaemia found in man as a result of chronic haemorrhage (Whitby and Britton 1950). This may be explained in part by the striking increase in the number of reticulocytes which are larger than mature red cells found in the peripheral blood of rabbits and sheep, but not in man, in response to blood loss of this nature.

The hypochromic feature of the anaemia associated with F. hepatica infections indicates that some disturbance in iron metabolism was probably a contributory factor in increasing its severity. The total quantity of iron in the body of these rabbits would normally be expected to be about 100 mg. of which perhaps 30 mg. was present in the iron stores as ferritin and haemosiderin. This iron can be drawn upon in times of need, but when the iron content of the body isaltered by some cause such as blood loss, balance is re-established mainly by changes in the amount of iron absorbed by the gut (McCance and Widdowson 1938). Assuming that the faecal iron excretion represents the amount of iron lost from the body each day (i.e. about 0.3 mg.) it would be expected that a similar amount of dietary iron would be absorbed to maintain the iron stores of these animals. Since the average daily ingestion of iron was about 13 mg., these rabbits would have had to absorb less than 3% of their dietary iron intake in order to make good excretory losses. Little

data is available regarding the ability of the rabbit to absorb dietary iron, but it is known that man and rat normally absorb 5 - 10% of iron ingested (Moore and Dubach 1951; Bannerman, O'Brien and Witts 1962). It would therefore seem reasonable to conclude that in these animals the dietary iron would readily meet the normal needs of the body.

However the fluke-infected rabbits lost about ten times more iron in the faeces each day than the controls, and although it is well established that iron absorption is increased in iron deficiency states and when erythropoiesis is stimulated, it seems improbable that these animals were able to increase dietary iron absorption to such an extent as to fully compensate for the large iron loss. Most evidence to date suggests that fluke-infected animals obtain the extra iron required for haemoglobin synthesis at the expense of the iron stores. Sinclair (1965) noted a reduction in marrow haemosiderin in sheep harbouring only 100 parasites. It is interesting to note that in these sheep, serum iron levels did not fall until 21 weeks post-infection (i.e. about 12 weeks after the flukes had entered the bile ducts) confirming that in the early stages the anaemia is not associated with a low level of serum iron. Since a fall in serum iron is characteristic of iron deficiency, it is clear that at some point in time the iron stores become depleted to such an extent that the increased demands of the marrow cannot be met from the iron stores and iron may then become the limiting factor in erythropoiesis.

Although the hypochromia observed in these rabbits was not particularly marked, P.C.V. values of infected rabbits fall further between about 3 and 5 months post-infection and this is associated with a significant increase in the magnitude of the intestinal blood and hence iron loss over this period (Section 4). These findings go far in explaining the significant decrease in MCHC values noted in fluke-infected rabbits (Urquhart 1955) and sheep (Jennings and Armour 1969) as anaemia develops. It would therefore appear that the increasing hypochromia of the anaemia associated with the disease is due to the substantial and increasing drain on the animal's iron stores, caused by the marked loss of iron into the gastro-intestinal tract. It is noteworthy that the rabbit in common with rat (Weintraub, Conrad and Crosby 1965) mouse and guinea pig (Conrad, Weintraub, Sears and Crosby, 1966) has no significant ability to absorb haemoglobin iron, whereas man (Walsh, Kaldor, Brading and George 1955) dog (Weintraub, Weinstein, Huser and Rafal 1968) and sheep (Clark, Kiessel and Goby 1962) have such an absorption mechanism. Thus in some animals ^{59}Fe-labelled erythrocytes provide a useful tool for quantitative measurement of gastro-intestinal blood loss. This inability of the rabbit to reabsorb the iron lost into the gut must undoubtedly be a contributory factor in causing a more rapid depletion of iron stores and hence increasing the severity of the anaemia associated with the disease.

SUMMARY

(1) Erythrocytes labelled with ^{51}Cr were used to measure the loss of red cells from the circulation into the gut of normal and fluke-infected rabbits. The results showed a striking difference between the normal and infected animals in the magnitude of this leak, suggesting that blood loss caused by the parasites is the principle factor in the aetiology of the anaemia associated with fascioliasis.

(2) The disappearance of ^{59}Fe from the plasma and its subsequent incorporation into the red cells was studied in normal and fluke-infected rabbits. Loss of isotope into the gut was used to quantitate the faecal iron loss. The results again showed a striking difference between the two groups and it was concluded that intestinal iron loss could be of sufficient magnitude to increase the severity of the anaemia associated with the disease.

(3) The ability of the rabbits to reutilise iron passed into the gut was estimated from the faecal excretion of ^{51}Cr and ^{59}Fe after simultaneously tagging the erythrocytes with these isotopes. It was found that both normal and infected animals were unable to utilise such iron. This was confirmed by following the faecal excretion of an oral dose of lysed cells labelled with ^{51}Cr and ^{59}Fe.

SECTION 3

SIMULTANEOUS MEASUREMENT OF THE LOSS OF RED CELLS AND PLASMA INTO THE GUT OF NORMAL AND FLUKE-INFECTED RABBITS AND THE EFFECT OF ANTHELMINTIC TREATMENT ON THE MAGNITUDE OF THESE LOSSES

INTRODUCTION

The results of the experiments described in the preceding sections of this thesis, show that rabbits chronically infected with F. hepatica lose amounts of plasma proteins and red cells into the gastro-intestinal tract likely to be of sufficient magnitude to account for the hypoproteinaemia and anaemia characteristic of the disease.

The simplest explanation of these findings is that such losses are of whole blood caused by the feeding activities of the flukes. Direct evidence of loss of whole blood requires the simultaneous determination of red cell and plasma losses by means of separate isotopic markers for each which are not reabsorbed from the gut and which preferably can be distinguished from each other by physical means.

The relationship between plasma and red cell loss was studied by the simultaneous use of rabbit albumin labelled with ^{95}Nb and red cells labelled with ^{51}Cr. Although elution of ^{51}Cr from the red cells and alteration of the biological life of albumin by labelling with ^{95}Nb limit the suitability of these labels for long term measurement of certain parameters of red cell and protein metabolism such as red cell life span and protein catabolic rate, since neither isotope is reabsorbed from the gut on oral administration, they provide very useful markers for quantitative measurement of enteric red cell and protein loss.

An experiment was therefore carried out to measure simultaneously the loss of plasma and red cells into the gut of normal and fluke-infected

rabbits over an initial period of 10 days. The rabbits were then treated with anthelmintic ("Zanil" 0.7 ml./kg.) and the measurements continued for a further period of 8 days. The anthelmintic treatment was carried out in order to establish if red cell and plasma loss stopped abruptly when the flukes were killed.

The effect of treatment on intestinal loss of plasma macro molecules and red cells was first studied separately in two smaller pilot experiments. In the first of these fluke infected and control rabbits were given an intravenous injection of ^{131}I-labelled P.V.P. and the disappearance of the label from the plasma followed for 7 days. All were then treated with anthelmintic and the plasma activity followed for a further period of 7 days. Estimates of movement of macromolecules into the gut were made by calculating faecal "clearances" of P.V.P. for each day of the study.

The effect of removal of the flukes on red cell loss was studied by injecting ^{51}Cr-labelled red cells into a further group of infected and control rabbits and following the blood, urine and faecal output of the isotope over an initial period of 12 days and then for a further period of 2 weeks after treatment.

MATERIALS AND METHODS

(i) Injection of ^{131}I-labelled P.V.P. and Blood Sampling

Three fluke-infected (50 metacercariae each 4 months previously) and 3 control rabbits were each injected with 1 ml. of labelled P.V.P. (350 μc.). The first blood sample was taken 18 hours after injection and subsequent samples once daily for a total period of 14 days. All rabbits were treated with anthelmintic on the seventh day of the experiment.

(ii) Injection of ^{51}Cr-labelled Red Cells and Blood Sampling

A measured volume of ^{51}Cr-labelled cell suspension containing about 200 μc. ^{51}Cr was injected into the marginal ear vein of 3 fluke-infected (50 metacercariae each 3 months previously) and 2 control rabbits, each rabbit receiving its own labelled red cells. The first blood sample was withdrawn 5 minutes after injection, a further 9 samples were collected at regularly spaced intervals over the first 9 days and thereafter (except for the day following treatment) one every other day for a further period of 16 days. All rabbits were treated with anthelmintic 12 days following injection of the labelled cells.

In the double labelling experiment, 40 ml. of blood was withdrawn from normal donor rabbits and incubated with 2.6 mc. ^{51}Cr as sodium chromate. Following removal of unbound isotope the labelled cells were reconstituted with 0.85% NaCl and measured amounts containing about 100 μc injected.

(iii) Labelling of Albumin with ^{95}Nb

Rabbit albumin was labelled with ^{95}Nb as previously described and unbound niobium removed by passing the preparation through an ion-exchange resin. Prior to injection the labelled albumin was dialysed overnight and after centrifugation at 1500 r.p.m. for 30 mins., carefully weighed doses (about 5 ml.) containing about 25 µc. ^{95}Nb were injected.

(iv) Experimental Animals and Blood Sampling

Five infected (50 metacercariae each 4 months previously) and 5 normal rabbits were used for this study. Two infected rabbits received only ^{59}Cr-labelled red cells, 1 being killed at 6 and the other at 10 days post-injection and the fluke burden determined. Two of the control rabbits were injected only with ^{95}Nb-labelled albumin and removed from the experiment 10 days later. The remaining 3 infected and 3 control animals were each injected with both labelled red cells and albumin. These rabbits were treated with anthelmintic 10 days later, and the experiment continued for a further period of 8 days. The first blood sample was taken 5 minutes after injecting the labelled cells and thereafter one sample was removed daily for a total period of 18 days.

(v) Collection of Urine and Faeces and Measurement of Radioactivity

In all experiments described in this section of the thesis, urine and faeces were collected as previously described and samples made up to 5 ml. for counting. One ml. aliquots of plasma and blood were pipetted into counting tubes and diluted to 5 ml. Suitable aliquots of standard solutions of all labelled preparations were assayed

at regular intervals and corrections for radioactive decay based on the activities of these solutions. In the double labelling experiment, count rates of samples of blood, urine and faeces were determined first on the ^{95}Nb and then on the ^{51}Cr photopeak. The ^{95}Nb "overlap" at the ^{51}Cr setting was accounted for by applying a correction factor calculated from the count rate of the ^{95}Nb standard at its own relative to that at the ^{51}Cr setting. It was thus possible to achieve complete separation of these isotopes at their respective settings.

RESULTS

(A). EFFECT OF ANTHELMINTIC TREATMENT ON MACROMOLECULAR LEAK IN NORMAL AND FLUKE-INFECTED RABBITS

Three fluke-infected and three control rabbits were each injected with 350 μc of labelled P.V.P. and faeces and plasma collected and assayed for radioactivity as described above.

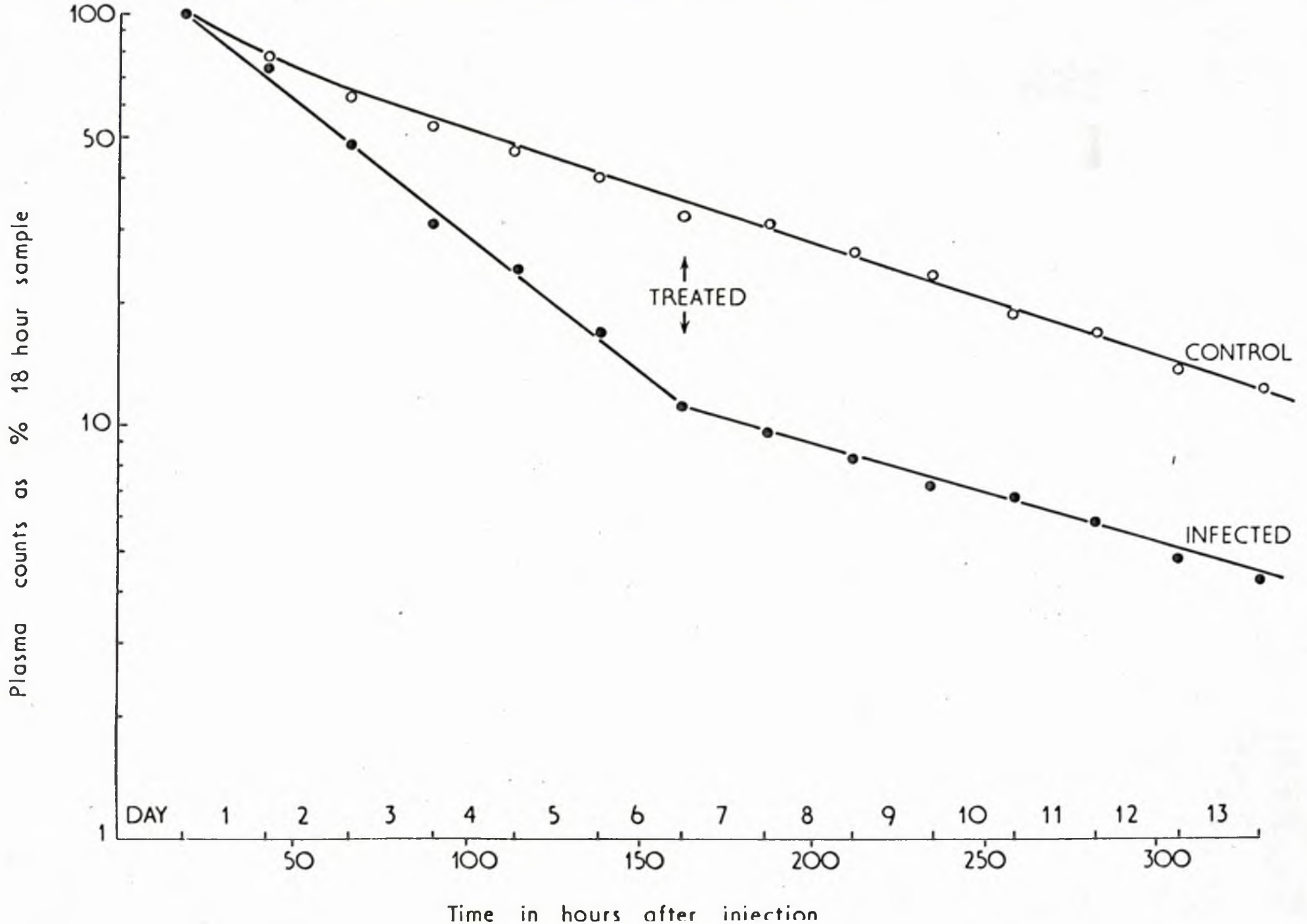
The radioactivity of each plasma sample was expressed as a percentage of the 18 hour post-injection value and plotted semi-logarithmically against time. Mean curves obtained for each group are shown in Fig. 15. Over the first 7-day period, the mean half-life of the preparation in the infected rabbits was 56 hours (S.D. 12) while in the controls, it was 104 hours (S.D.6). However, over a similar period following treatment the corresponding values were 117 hours (S.D.16) and 108 hours (S.D. 6) respectively, demonstrating a dramatic change in the rate of disappearance of the label from the plasma of the infected group. Half-life values of individual rabbits are shown in Table 25, (N.B. Rabbit No. 263 died following treatment and was not included in Fig. 15).

(i) Faecal Excretion of ^{131}I-labelled P.V.P. before and after Treatment

Plasma "clearances" were calculated as previously described for each day of the experiment and mean results shown in Table 25. Prior to treatment, the average daily "clearance" value in the infected group was 12.1 ml. (S.D. 3.8) but by 7 days post-treatment this had decreased to 1.8 ml. (S.D. 0.4), a figure not significantly different from that of

FIGURE 15

^{131}I - PVP ANTHELMINTIC TREATED RABBITS



Plasma Half - life and Faecal "Clearance" of ^{131}I - Labelled P.V.P. in Normal and Fluke - Infected Rabbits Before and After Treatment with Anthelmintic

TABLE 25

	Rabbit No.	Apparent Half-Life(hr)		Daily Faecal Clearance(ml)	
		Pre-treatment	Post-treatment	Pre-treatment	7 days Post-treatment
CONTROL	110	103	115	1.1	1.7
	039	99	99	1.4	1.6
	203	110	110	1.6	0.7
	Mean	104	108	1.4	1.3
	S.D.	6	7	0.3	0.6
INFECTED	286	46	128	16.5	2.0
	258	69	106	9.4	1.5
	263	54	-	10.4	Died
	Mean	56	117	12.1	1.8
	S.D.	12	16	3.8	0.4
	P	< 0.01	N.S.	< 0.01	N.S.

the normal rabbits. Daily "clearances" obtained for each rabbit are shown in Appendix C. The striking nature of this reduction in macromolecular leak following treatment is illustrated in Fig. 16. Although the average "clearance" of P.V.P. in the infected animals was reduced to less than half by 24 hours following treatment, this rate of decline was not maintained and only after about 5 or 6 days post-treatment did the "clearances" of the infected group fall to the control level.

Conclusions

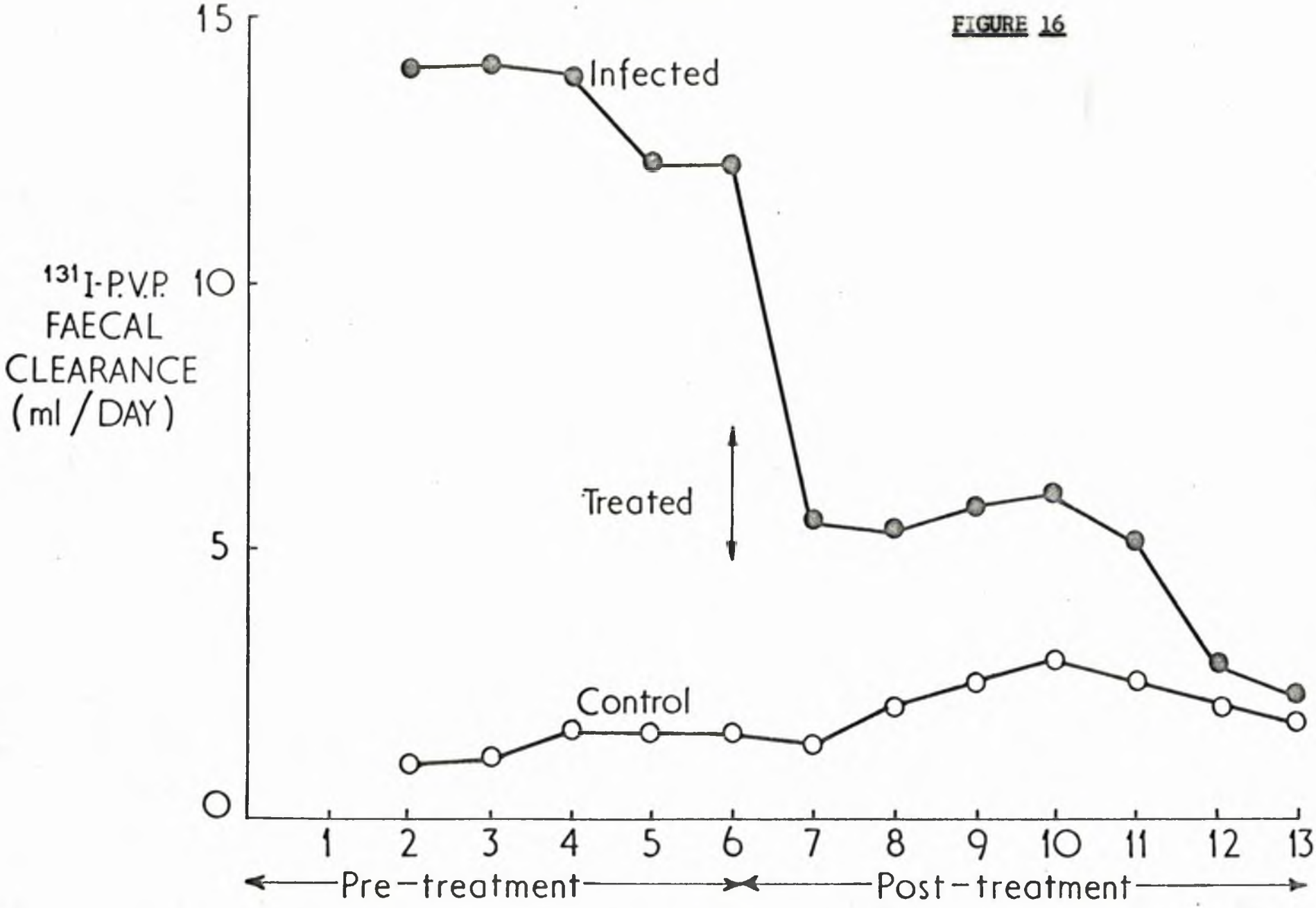
At autopsy, no flukes were found in the rabbits which survived treatment. Thus the results of this experiment demonstrate in a very dramatic fashion the effect of removal of the flukes on the movement of plasma macromolecules into the gut of infected rabbits and lend further support to the theory that the major cause of the hypoproteinaemia in chronic fascioliasis is the loss of plasma into the gastro-intestinal tract, due to the presence of the adult flukes in the bile ducts. It should however be pointed out that because of the inappetence of all animals during the 48 hours immediately following treatment, the marked decrease in the "clearance" figures of the infected rabbits was partly due to unusually low faecal output over this period. On the other hand, the slight increase observed between the 2nd and 4th day of this period reflects the return of these animals to normal feeding.

(B) EFFECT OF TREATMENT ON THE PASSAGE OF ^{51}Cr-LABELLED ERYTHROCYTES INTO THE GUT OF NORMAL AND FLUKE-INFECTED RABBITS

In order to determine whether removal of the flukes would similarly reduce the intestinal loss of red cells suffered by fluke-infected

EFFECT of ANTHELMINTIC TREATMENT on the MAGNITUDE of MACROMOLECULAR LEAK in NORMAL and FLUKE-INFECTED RABBITS

FIGURE 16



rabbits, ^{51}Cr-labelled erythrocytes were injected into 3 infected and 2 normal animals and the blood levels and faecal and urinary output of isotope followed over an initial period of 12 days and then for a further period of 2 weeks as previously described.

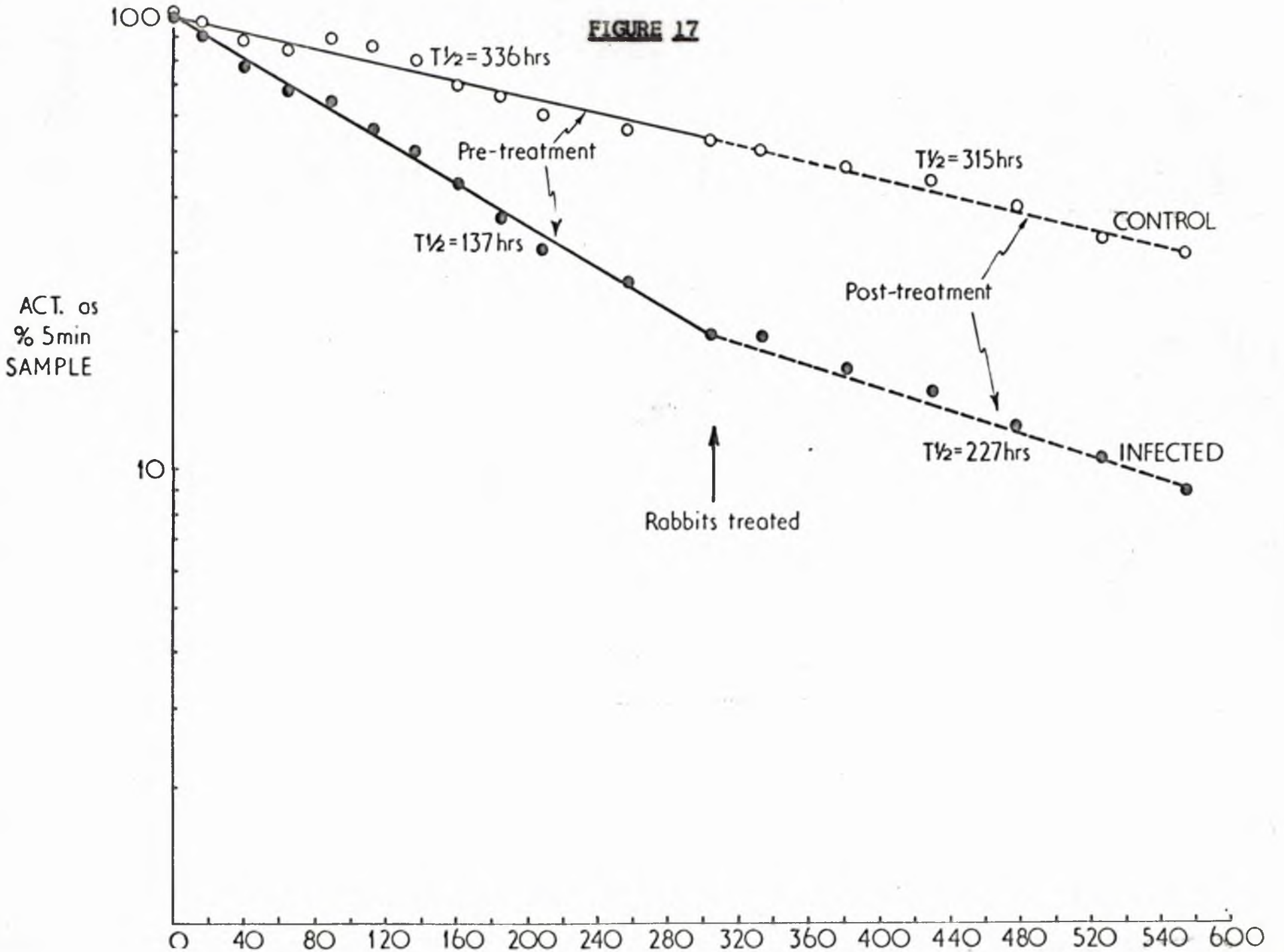
(i) Persistence of Labelled Cells in Fluke-Infected and Normal Rabbits Before and After Treatment

The radioactivity of each blood sample was expressed as a percentage of the 5 minute post-injection value, and from the haematocrit determination on each sample, the activity per ml. of packed red cells was calculated. A semi-log plot of both whole blood and red cell activity against time was made. Mean curves obtained for both are shown in Figs. 17 and 18 respectively. It is clear that after treatment, the survival of the labelled cells in the circulation of the infected rabbits was considerably increased relative to the pre-treatment period. Whereas in the infected rabbits the half-life of the label expressed in terms of both whole blood and packed red cells was significantly greater during the post-treatment period ($P < 0.01$) no such difference was apparent in the control animals. Half-life values of each rabbit pre- and post-treatment are shown in Table 26.

It was previously pointed out (Section 2) that comparison of half-life values of ^{51}Cr-labelled red cells in different groups of animals is only valid if it can be assumed that the circulating red cell volume of each animal remains constant from day to day. Although the red cell volumes of the animals used in this study were not determined at the end

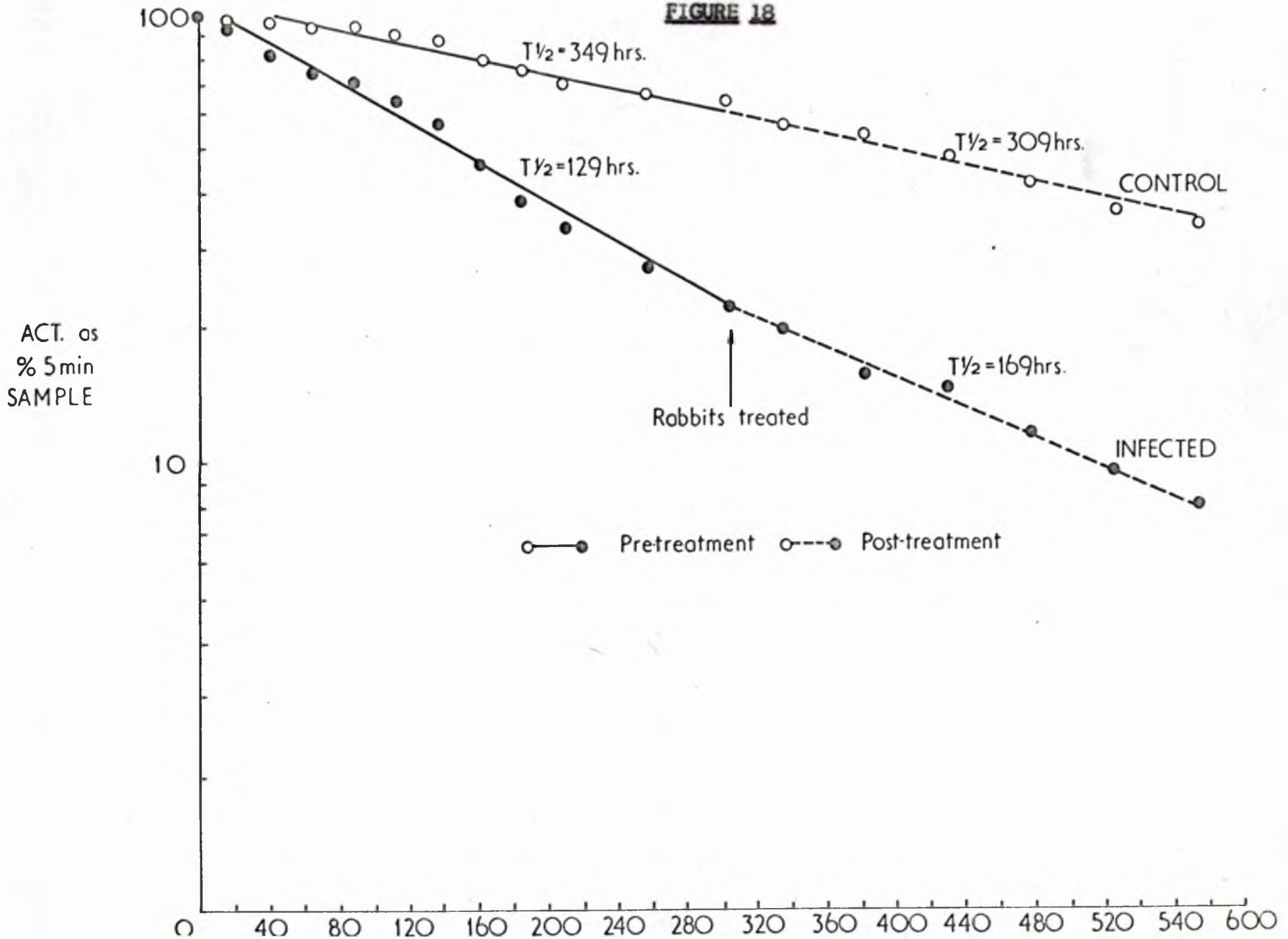
EFFECT of ANTHELMINTIC TREATMENT on the TURNOVER of ^{51}Cr-LABELLED ERYTHROCYTES MEAN BLOOD DISAPPEARANCE CURVES

FIGURE 17



EFFECT of ANTHELMINTIC TREATMENT on the TURNOVER of ^{51}Cr-LABELLED ERYTHROCYTES MEAN R.B.C. DISAPPEARANCE CURVES

FIGURE 18



EFFECT OF ANTHELMINTIC TREATMENT ON THE TURNOVER OF ^{51}Cr - LABELLED ERYTHROCYTES IN NORMAL AND FLUKE - INFECTED RABBITS

TABLE 26

Rabbit No.	PRE - TREATMENT					POST - TREATMENT				
	Mean P.C.V.	Half - life(hr) Whole Blood	Half - life(hr) Red Cells	Mean Faecal Clearance Whole Blood(ml/day)	Mean Faecal Clearance Red Cells (ml/day)	Mean P.C.V.	Half - life(hr) Whole Blood	Half - life(hr) Red Cells	Mean Faecal Clearance Whole Blood(ml/day)	Mean Faecal Clearance Red Cells (ml/day)
423	40	340	372	0.06	0.03	39	330	324	0.15	0.05
424	39	332	326	0.06	0.02	39	300	294	0.07	0.02
Mean	39.5	336	348	0.06	0.03	39	315	309	0.11	0.04
S.D.	0.7	6	33	-	0.01	-	21	21	0.06	0.02
P_C	N.S.	N.S.	N.S.	N.S.	N.S.					
320	29	150	150	3.59	1.06	39	236	184	0.81	0.27
346	27	154	136	5.02	1.61	38	234	188	0.69	0.22
438	31	106	102	7.85	2.38	43	212	136	1.96	0.65
Mean	29	13	129	5.49	1.68	40	227	169	1.15	0.38
S.D.	2	27	25	2.17	0.66	2	13	29	0.70	0.24
P_I	<0.01	<0.01	<0.01	<0.05	<0.05					
P	<0.01	<0.01	<0.01	<0.05	<0.05	N.S.	<0.02	<0.02	N.S.	N.S.

P_C and P_I = Significance of Difference in Controls and Infected respectively before and after Treatment.

of the experiment, it would seem likely, because of the marked increase in red cell production previously demonstrated in infected rabbits, that removal of the flukes would result in some increase in the red cell volume of these animals. Indirect evidence of such an increase is apparent from the striking difference in the venous haematocrit of each infected rabbit determined at the beginning and end of the experiment (Table 26). Thus, prior to injection of the labelled red cells, the mean P.C.V. of the infected animals was 29% (S.D. 2) while that of the controls was 39.5% (S.D. 0.7), and whilst in the former group the mean P.C.V. had increased to 40% (S.D. 2) by 14 days following treatment, no such difference was noted in the control animals. The rapidity with which the venous haematocrit of the infected rabbits increased following treatment is illustrated in Figs. 19 and 20.

Since the P.C.V. of the infected animals increased progressively following treatment, new unlabelled cells must have diluted the labelled cells already present in the circulation of these animals at a more rapid rate than in the controls, and as a result, the half-life values of the labelled cells in the infected rabbits are underestimated. Clearly, this effect will be much more apparent in curves plotted as activity/ml. of packed red cells than as whole blood and this is well illustrated in Figs. 17 and 18. Thus although the rate of disappearance of the labelled cells from the circulation of the infected rabbits is still significantly greater than in the controls following removal of the flukes, this was most likely caused not so much by a continuing loss of cells, but by a more rapid "dilution" of labelled by unlabelled cells.

(ii) Faecal Excretion of ^{51}Cr Before and After Treatment

Faecal "clearances" of whole blood and red cells were calculated as previously described for each day of the experiment and the figures obtained for individual rabbits presented in both tabular and graphic form in Appendix C. Average daily values are shown in Figs. 19 and 20 respectively. Following treatment loss of isotope into the gut of infected animals fell abruptly to about 15% of the average pre-treatment value, and apart from a slight increase between 3 and 7 days post-treatment, decreased further until after about two weeks, there was no significant difference in the values obtained for the two groups. The average daily pre- and post-treatment blood and red cell "clearances" are shown in Table 26. Although the average post-treatment figures of the infected group were considerably greater than those of the controls, it should be borne in mind that they are "average" values and as such do not show the decline in the magnitude of the blood loss with time.

Conclusions

It is clear that removal of the flukes results in a dramatic reduction in the passage of labelled red cells into the gut of fluke-infected rabbits, and an associated return of the venous haematocrit of these animals to normal levels. It is interesting to note however that although the flukes were probably killed within two or three days following treatment, there was still a marked loss of isotope into the gut of the infected animals during the first 10 days of the post-treatment period.

Effect of Anthelmintic on the turnover of ^{51}Cr-Labelled Erythrocytes Mean P.C.V. and Blood Clearances Pre- and Post-treatment.

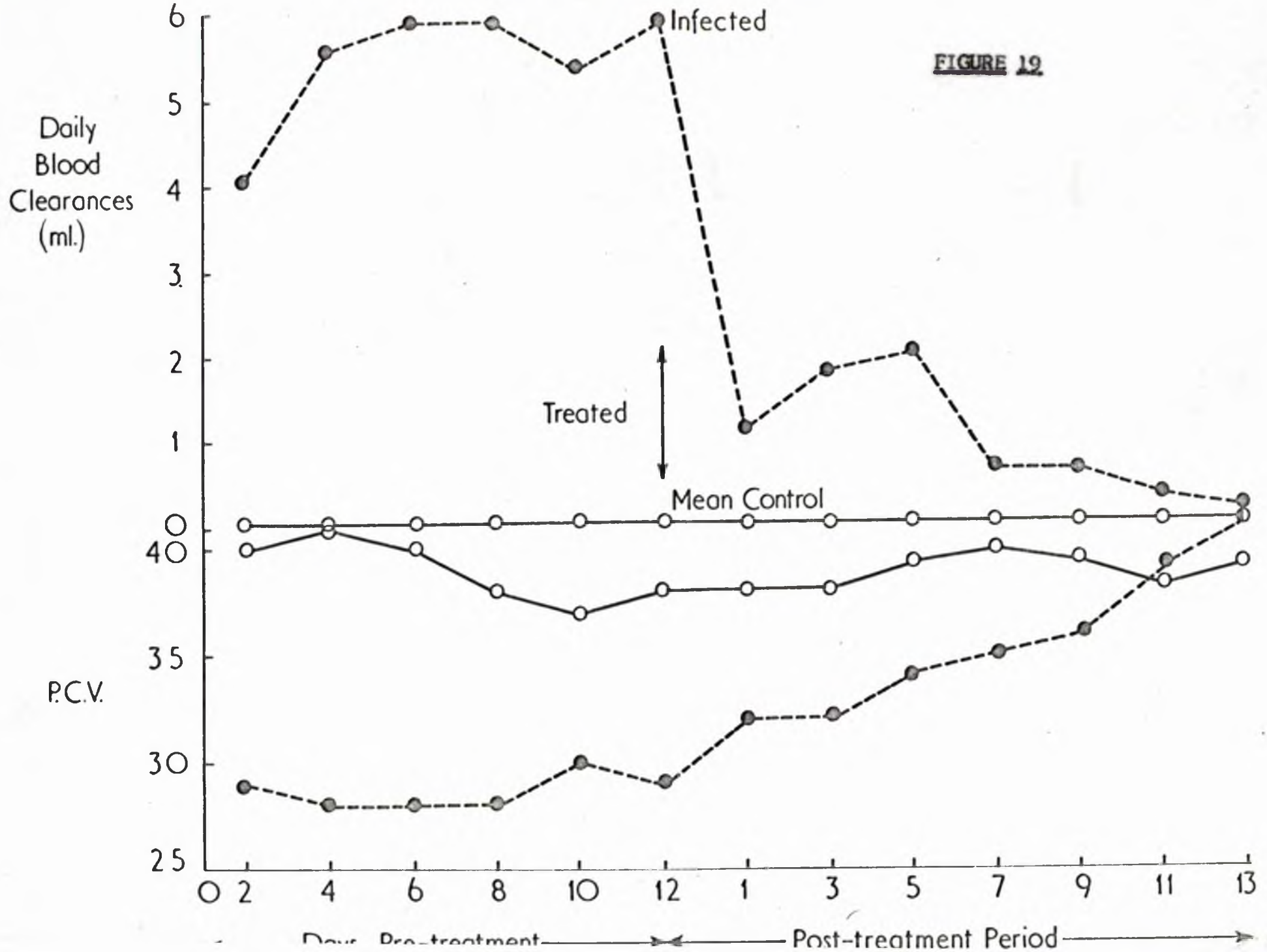
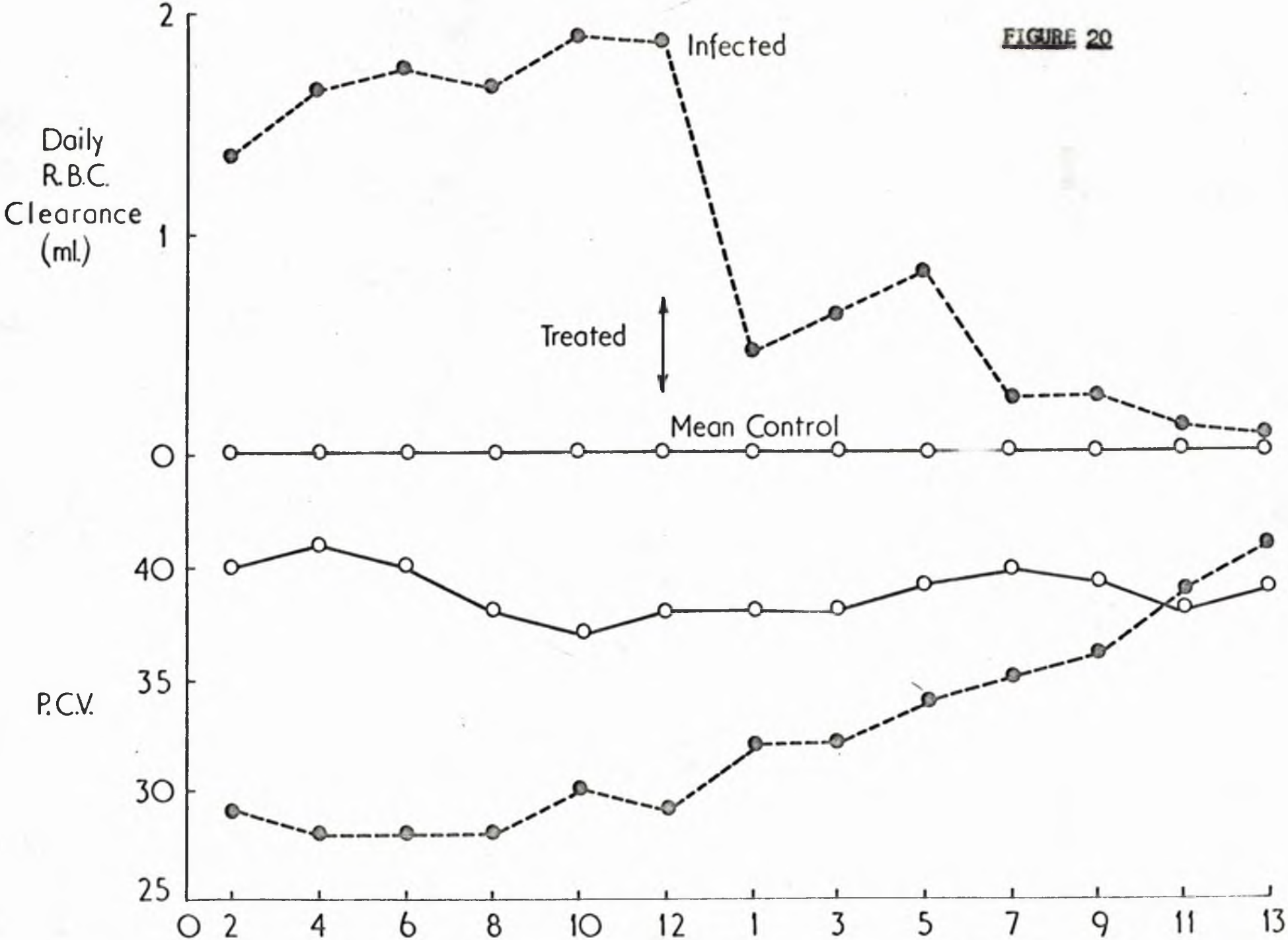


FIGURE 19

Effect of Anthelmintic treatment on the turnover of ^{51}Cr-Labelled Erythrocytes

Mean P.C.V. and Red Cell Clearances Pre-and Post-treatment

FIGURE 20



(C) SIMULTANEOUS MEASUREMENTS OF INTESTINAL RED CELL AND PROTEIN LOSS BEFORE AND AFTER TREATMENT WITH ANTHELMINTIC

Three fluke-infected and 3 control rabbits were each injected with ^{95}Nb-labelled albumin and ^{51}Cr-labelled red cells at the same time, and the blood levels and faecal and urinary output of both isotopes followed for an initial period of 10 days and then for a further period of 8 days after treatment with anthelmintic. In addition, 2 control and 2 infected rabbits were respectively injected with only labelled albumin and red cells and the blood, faecal and urinary levels of isotope followed as previously described.

(1) Persistance of ^{51}Cr-labelled Red Cells and ^{95}Nb-labelled Albumin in the Circulation of Fluke-infected and Normal Rabbits

The radioactivity of each blood sample was expressed as a percentage of the 5 minute post-injection value, and from the venous haematocrit determination on each sample, the activity per ml. of red cells was calculated. A semi-log plot of blood and red cell activity against time was made. As in the previous experiment, the rate of disappearance of labelled cells (expressed in terms of activity/ml. R.B.C.) from the circulation of the fluke-infected rabbits (mean half-life 105 hours S.D. 27) was greater than in the controls (mean half-life 200 hours S.D. 12) during the pre-treatment period. However, during the 8-day period following treatment, whereas the half-life of the labelled cells in the control animals remained unaltered, that of the infected group increased to 145 hours (S.D. 9).

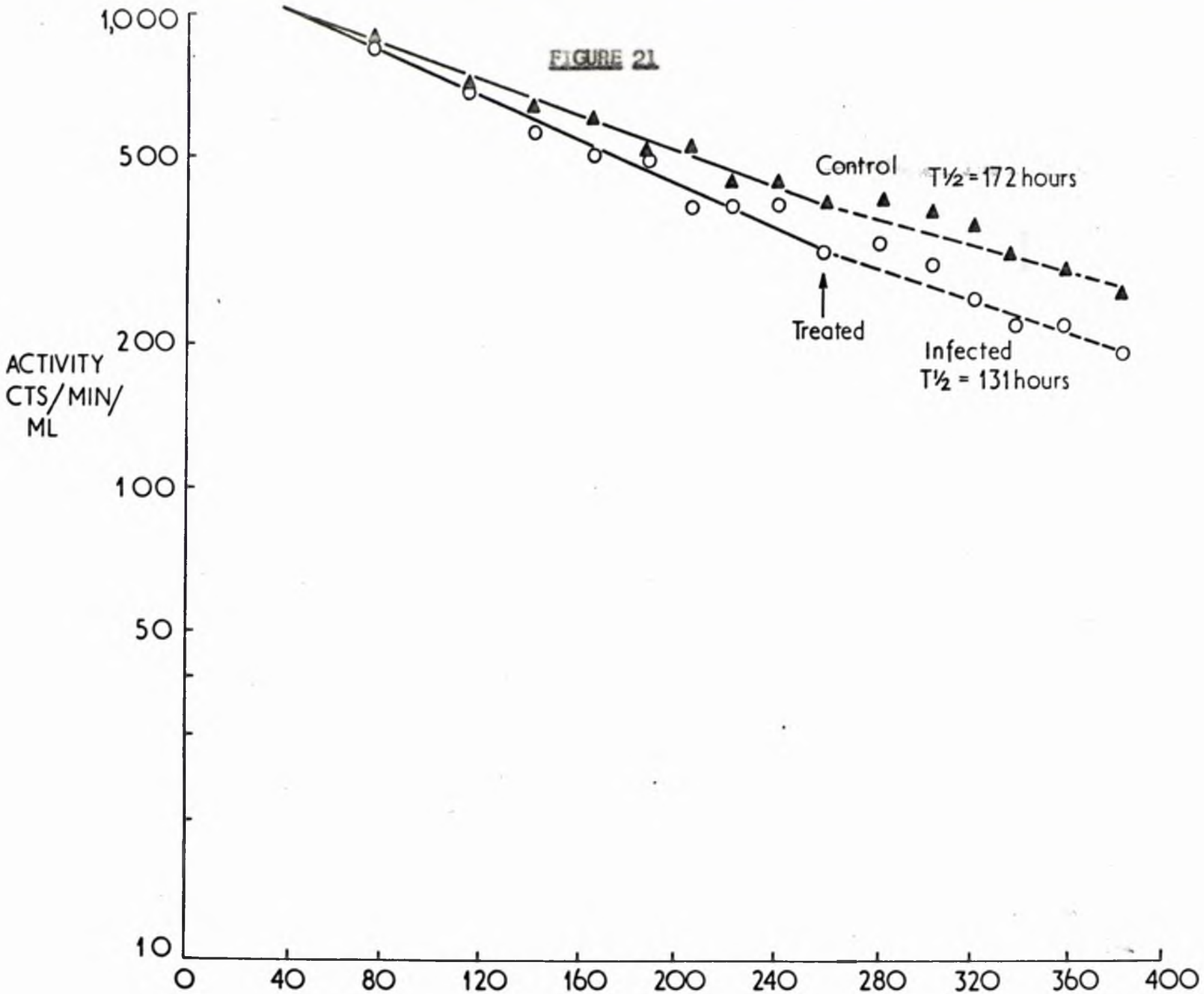
The radioactivity of each plasma sample was plotted semi-logarithmically against time and the mean curves obtained for the infected and control group shown in Fig. 21. The label was eliminated from the plasma very rapidly during the first two days following injection, but thereafter a more gradual exponential decline in activity is apparent. The mean half-life of the preparation was 131 hours (S.D. 25) in the infected rabbits, and 172 hours (S.D. 34) in the controls. This difference was not statistically significant ($P > 0.05$). The initial more rapid disappearance of the label from the plasma was not followed by rapid urinary excretion of the isotope, suggesting that marked tissue retention occurred. It is thus possible that the isotope subsequently re-entered the circulation, bound to some other molecule, thereby increasing the half-life of the preparation. The ^{95}Nb plasma radioactivity was completely precipitable with T.C.A. within the limits of experimental error.

Matthews and Gartside (1965) noted that the distribution of intravenously injected ^{95}Nb in rats was very similar to that of ^{131}I-labelled albumin and concluded that the isotope probably became protein bound. These authors also fractionated plasma samples by sodium sulphate precipitation and found that the radioactivity was divided between the albumin and gammaglobulin fractions. These observations do not detract from the usefulness of the label for detecting and probably quantitating plasma loss into the gut.

(ii) Faecal Excretion of ^{51}Cr and ^{95}Nb

By comparing the activity of each isotope in the daily faecal

^{131}I-LABELLED ALBUMIN PLASMA DISAPPEARANCE CURVE



collections with that of the appropriate blood and plasma samples, the total amount of whole blood, red cells and plasma which had entered the gut over the collection period were calculated. The average daily "clearance" values obtained during the initial 10 days of the experiment, together with those of the 6 - 8 day period following treatment are shown in Table 27. Mean values were calculated using only those rabbits which were injected with both labelled cells and protein. Daily blood, red cell and plasma "clearances" of each rabbit are shown in Appendix C. It is apparent from Table 27 that the "background" protein leak as manifested by the plasma "clearance" in normal animals both pre- and post-treatment, and in the post-treatment period in infected animals was much more substantial than the background blood loss. This is to be expected since a normal leakage of plasma protein into the gastrointestinal tract occurs in all animals. One of the control rabbits had enteritis during most of the experiment and thus plasma "clearances" were unrealistically high for a "normal" animal. However, since the "clearances" of the remaining 3 control rabbits were very similar, it would be reasonable to conclude that about 5 ml. of plasma normally passes into the intestinal tract of the rabbit each day.

It is also clear from Table 27 that by about one week following treatment with anthelmintic, the loss of red cells and plasma into the gut of the infected rabbits had fallen virtually to control level, thus confirming the results of the previous treatment experiments.

^{95}Nb-Labelled Albumin/ ^{51}Cr-Labelled Erythrocytes Experiment in Rabbits

TABLE 27

AVERAGE DAILY FAECAL "CLEARANCE" OF BLOOD, RED CELLS AND PLASMA(ML.)

	RABBIT NO.	PRE-TREATMENT BLOOD	PRE-TREATMENT R.B.C.	PRE-TREATMENT PLASMA	(6-8 DAYS) POST-TREATMENT BLOOD	POST-TREATMENT R.B.C.	POST-TREATMENT PLASMA	FLUKES RECOVERED
INFECTED	036	16.2	3.90	25.1	2.9	0.71	5.7	-
	182	5.8	1.73	18.8	0.6	0.20	5.8	3(Weak)
	712	5.9	1.61	36.8	1.2	0.23	10.4	15(Dead)
	332	11.5	4.30	-	-	Killed	-	33
	314	15.6	6.43	-	-	Killed	-	37
	Mean	9.3	2.41	26.9	1.6	0.38	7.3	
	S.D.	5.9	1.29	9.1	1.2	0.29	2.7	
CONTROL	† 384	0.13	0.04	10.4	0.11	0.04	13.1	
	027	0.06	0.02	5.1	0.11	0.03	5.2	
	X	0.13	0.04	a	0.20	0.08	a	
	055	-	-	4.21	-	Not Treated	-	
	057	-	-	5.9	-	Not Treated	-	
	Mean	0.11	0.03	7.8	0.14	0.05	9.2	
	S.D.	0.04	0.01	3.8	0.05	0.03	5.6	
	P	<0.05	<0.05	<0.05	N.S.	N.S.	N.S.	

† Enteritis.

a Activity too low for accurate measurement.

Conclusions

It was previously shown (Section 1) that normally about 20% of the intravascular pool of albumin is catabolised daily in the rabbit. Making the assumption that the mean plasma volume of the rabbits used in this experiment was about 80 ml., it would be expected that the equivalent of 16 ml. of plasma was catabolised daily in the control animals. Since the normal daily faecal "clearance" of plasma calculated from the ^{95}Nb plasma and faecal radioactivities was about 5 ml., it would appear that loss of plasma into the gut could account for at least 30% of the total daily catabolism of plasma proteins in the rabbit.

Similar results were obtained by Armstrong et al (1960) using perfused rabbit gut. It is also interesting to recall from the turnover studies of albumin and IgG in normal and fluke-infected rabbits (Section 1) that about 25% of the plasma volume (20 ml. of plasma) was lost into the gut of the infected group if it was assumed that the observed hyper-catabolism of these proteins was due entirely to increased enteric protein loss. In the present experiment, where plasma loss was directly estimated and not indirectly derived from the differences in catabolic rate, the mean daily plasma loss suffered by the infected rabbits was 27 ml. If the normal daily loss is subtracted from this figure the loss due to the presence of flukes is about 22 ml., which in these animals represents roughly 25% of their plasma volume. It would therefore appear that ^{95}Nb-labelled albumin provides a very useful label for quantitating loss of plasma proteins into the gastro-intestinal tract.

The results of this experiment also show that even when allowance is made for the normal enteric protein loss, the passage of plasma proteins into the gut is clearly much greater than can be accounted for by the whole blood loss and it would therefore appear that chronic liver fluke infection causes a loss to the host of plasma over and above that expected from a loss of whole blood.

DISCUSSION

The effect of removal of the flukes on the magnitude of the leak of red cells and plasma macromolecules lends further support to the theory that the major cause of the anaemia the hypoproteinaemia in fascioliasis is the loss of red cells and plasma into the gastro-intestinal tract due to the presence of the adult flukes in the bile ducts. Just how these blood constituents pass into the gut has not been directly established, but the results of this and the preceding sections can only be interpreted in one or both of two ways. The simplest explanation is that the fluke sucks blood from the vessels in the wall of the bile ducts. However, it is also known that when the flukes enter the bile ducts, the epithelium becomes folded into crypt-like formations and these are covered by a slimy secretion. Flukes move about over this "compound" epithelium and sometimes completely remove parts of it, and it is therefore possible that the spines, eggs and suckers of the parasite mechanically lacerate the wall of the bile duct thereby causing a constant haemorrhage. Either or both of these mechanisms will result in the passage of labelled molecules into the gut via the bile.

There is now much evidence in favour of the theory that the parasite is haematophagic. Urquhart (1955) noted that the flukes adhere by their suckers to the mucosa of the bile duct, and that their caeca were often filled with intact red cells and leucocytes. This author also examined bile from normal and fluke-infected rabbits for occult blood, and showed that only bile removed from parasitised animals gave a positive benzidine

reaction. More recently, Todd and Ross (1966) confirmed that the black caecal contents of flukes removed from cattle and sheep contained appreciable amounts of haemoglobin or its breakdown products and by comparing the iron and copper contents of blood, bile, bile duct epithelium liver and caecal contents, concluded that the liver fluke is almost entirely haematophagic. This view is also shared by Symons and Boray (1967) who showed by histological examination of the site of fluke attachment to the bile ducts of sheep, that the mucosa was often missing and replaced by a blood clot. However Dawes and Hughes (1964) although admitting that the flukes are actively engaged in feeding on hyperplastic epithelium, are careful to point out that "there was no trace of blood anywhere in the picture, although dark pigmented materials are seen in the caeca in copious amounts". Perhaps examination of these materials would have led these authors to a different conclusion.

It is unlikely that the blood-sucking activities of the parasite are solely responsible for the clinical picture associated with the chronic fascioliasis. The findings of Murray (1968) are of considerable relevence in this respect. This author carried out electron microscope studies on tissues from cattle infected with O. ostertagi and noted the presence of electron dense material between epithelial cells lining the gastric mucosa. On the basis that the electron density of this material was similar to known protein-containing structures within cells such as zymogen granules, Murray concluded that protein leaking through the hyperplastic gastric mucosa, together with the mechanical damage done by the parasite itself to

the mucosa was the probable cause of the hypoalbuminaemia associated with this disease. Presumably a similar breakdown of the junctions between bile-duct epithelial cells could occur in chronic fascioliasis.

Since massive haemorrhage is not a feature of the histology of parasitised bile ducts, it would seem reasonable to suggest on the basis of the above findings, together with the results obtained from simultaneous measurements of plasma and red cells loss into the gut, that liver fluke infection causes a loss to the host of whole blood very probably due to the feeding activities of the parasite, and over and above this, a loss of plasma protein presumably occurring as a result of damage to the bile ducts.

SUMMARY

(1) The effect of anthelmintic treatment on the intestinal loss of plasma macromolecules and red cells was studied in normal and fluke-infected rabbits. Following treatment, the rate of disappearance of ^{131}I-labelled P.V.P. and ^{51}Cr-labelled red cells from the circulation of the infected animals decreased dramatically, and in all cases this was associated with a rapid reduction in faecal excretion of these isotopes.

(2) Simultaneous measurements of plasma and red cell loss were made before and after anthelmintic treatment using ^{95}Nb-labelled albumin and ^{51}Cr-labelled red cells in a third group of normal and infected rabbits. By about 1 week following treatment, the loss of both cells and plasma into the gut had fallen virtually to control level.

These results are consistent with the theory that the anaemia and hypoproteinaemia associated with chronic fascioliasis are caused by the feeding activities of the adult flukes in the bile ducts.

SECTION 4

A STUDY OF THE ONSET AND DEVELOPMENT OF THE ANAEMIA AND HYPOALBUMINAEMIA ASSOCIATED WITH F. HEPATICA INFECTIONS IN THE RABBIT

INTRODUCTION

All the experiments described in the preceding sections were carried out on rabbits harbouring populations of adult parasites. No information is yet available however on plasma protein and red cell turnover during the migratory phase of the parasites.

It is generally considered that while wandering in the liver, the young flukes leave tracts through the parenchyma by rupturing hepatic cells and ingesting their contents. Since haemorrhage also occurs from the liver sinusoids, the spaces left in the wake of the flukes are usually filled with erythrocytes in addition to cell debris lymphocytes and macrophages. In the latter stages because of the rapid growth of the parasite during migration, the tracts produced by their burrowing activities cause more extensive damage to the liver.

Since the liver is the sole organ of albumin synthesis, and is also important as a store for iron, vitamin B_{12} and folic acid, it is possible that the anaemia and hypoalbuminaemia previously noted in chronically infected rabbits developed not only as a result of increased breakdown or loss of these blood constituents into the gut when the flukes entered the bile ducts, but from impairment to albumin and red cell production caused by hepatic damage and that this might be more important in the earlier stages of infection. Furthermore the importance of the haemorrhagic tracts produced by the burrowing activities of the young flukes must also be assessed.

It is now well established that the parasites enter the bile ducts

of the rabbit between 6 and 8 weeks after infection, and that a short time thereafter begin egg laying. For some time after entering the biliary system both the size and egg output of the flukes continue to increase and it therefore seems likely that their nutritional demands will also substantially increase. Although it has been consistently demonstrated that both the rate of degradation of plasma proteins and red cells and their loss into the gastrointestinal tract are very much greater in 10 - 12 week infection rabbits than in controls no comparison has been made with the situation which exists at say 3 months post-infection i.e. it is necessary to establish whether albumin catabolism and blood loss for example increase as the size and metabolic activity of the flukes increase. Thus comparison of the metabolism of these blood constituents at different stages of infection is necesaary to assess more precisely the pathogenic effects of the parasite on the host.

In order to study the onset and development of the albumin catabolism and anaemia, it is necessary to measure simultaneously the turnover of labelled albumin and red cells from the time of infection until after the flukes have reached maturity. In this way, it is possible to establish at what stage in the disease liver fluke infection causes increased degradation, and whether the changes in blood composition observed in infected animals (e.g. P.C.V., red cell volume, and serum albumin concentration) coincide with the altered kinetics. Although the rates of albumin and red cell production cannot be measured directly an assessment may be made from a knowledge of both the rate of degradation

or loss and the amount of each constituent present in the circulation at various times throughout the study. It should also be possible from the rate and route of isotopic excretion at each stage of infection to establish whether for example, the onset and increasing severity of the anaemia is directly related to loss of erythrocytes into the gut, or whether perhaps excessive haemolysis during the migratory phase plays any part. Furthermore, by comparing degradation rates and losses into the gut at the time the flukes become established within the bile ducts with those obtained 8 weeks later in the same animals some assessment of the relative demands of the parasite on the host at these times may be made.

Studies on the onset and development of albumin hypercatabolism and anaemia were carried out by simultaneously injecting ^{125}I-labelled albumin and ^{51}Cr labelled red cells (these isotopes can be measured in the same sample because of differences in the energy spectrum of their radiations) and following the blood levels and faecal and urinary output of each isotope during three phases of the disease process. The first two experiments covered the period from the time of infection until the flukes reached maturity in the bile ducts, while in the third, measurements of albumin and red cell degradation and loss into the gut between 18 and 20 weeks post-infection were compared to the values obtained between 8 and 10 weeks.

Estimates of the movement of plasma macromolecules into the gut were also made from studies on the faecal excretion of ^{131}I-labelled P.V.P. for a period of 10 weeks after infection in order to establish whether the onset of albumin hypercatabolism coincides with an increased faecal "clearance" of P.V.P.

Because of the possibility of a further increase in both albumin and red cell degradation or loss between 10 and 18 weeks post-infection, the magnitude of intestinal plasma and red cell loss was simultaneously estimated between 6 and 8 weeks post-infection (i.e. at the time of entry of the flukes into the bile ducts) and again at 18 weeks in the same infected rabbits using ^{95}Nb-labelled albumin and ^{51}Cr-labelled erythrocytes.

MATERIALS AND METHODS

(A) Albumin and Red Cell Turnover Studies in Rabbits Following Infection with F. hepatica. Simultaneous Use of ^{125}I-labelled Albumin and ^{51}Cr-labelled Red Cells

(i) Labelling of Albumin with ^{125}I

Labelling was carried out by the method of McFarlane (1958). In all experiments 10 ml. 2.5% rabbit serum albumin was trace labelled with 5 mc. $Na^{125}I$. Each rabbit was injected with a carefully weighed amount of labelled protein containing 350-400μc.

(ii) Labelling of Red Cells with ^{51}Cr

Blood samples containing about 2 ml. of packed red cells were withdrawn from each animal under study and incubated for 1 hour with 400 μc ^{51}Cr as sodium chromate. The labelled cells were then washed until free of unbound isotope and reconstituted approximately in proportion to the original haematocrit before injection.

(iii) Experimental Procedure

(a) Phase 1:- The Migratory Phase - Up to 38 days post-infection

Six rabbits were each injected with labelled protein and red cell suspensions at the same time. Four days later, 3 of these animals were each infected with 50 metacercariae and the experiment continued for a further period of 38 days. The first blood sample was taken 5 minutes after injection and subsequent samples collected daily during the first 4 days and thereafter every third day. In this, and all other experiments

involving the use of radioiodine, the drinking water of all animals was replaced with a solution containing inactive iodide.

(b) Phase 2:- Entry of Flukes into Biliary System and Establishment of Chronic Infection - Day 35 - 70 Post-infection

Five rabbits which had each received 50 metacercariae 5 weeks previously and 3 controls were similarly injected with labelled albumin and erythrocytes. The first blood sample was taken 5 minutes later, a further 3 samples collected at spaced intervals over the first 3 days, and thereafter one every otherday for a total period of 5 weeks.

(c) Phase 3:- A Study of Long-standing Infections - 18 - 20 Weeks Post-infection

The rabbits used in Phase 2 (with the exception of No. 307 which died in the interval), were again injected with labelled protein and red cells and the blood level of each isotope followed for 2 weeks. As in previous experiments, the first blood sample was withdrawn 5 minutes post-injection, a further 6 samples collected over the first 3days and thereafter one daily for 11 days.

(iv) Serum Protein Determinations

Total serum protein and individual fractions were estimated as previously described.

(v) Plasma and Red Cell Volume Estimations

The plasma and red cell volume of each animal was estimated from the radioactivity of the 5 minute samples of plasma and of whole blood corrected for venous haematocrit respectively by the isotope dilution

principle. Blood volume was calculated as the sum of the plasma and red cell volumes.

(vi) Albumin Turnover Calculations

The plasma volume together with the appropriate serum albumin concentration enabled the intravascular pool (CA) to be calculated. The determination of the extravascular pool was based on both the "equilibrium time method" described by Campbell et al (1956) and on the extrapolation procedure (Sterling 1951).

The catabolic rate of albumin was assessed from the slope of the final exponential of the plasma activity curve (Sterling 1951) and by calculating the fractional catabolic rate (K) i.e. the fraction of the intravascular pool degraded each 24 hours. K was calculated in two different ways, namely from the total radioactivity excreted every 24 hours divided by the total intravascular radioactivity (Campbell et al 1956) and from analysis of the plasma activity curve by the method of Matthews (1957).

(B) Estimation of the Movement of Plasma Macromolecules into the Gut of Rabbits Following Infection with F. hepatica - Use of ^{131}I-labelled P.V.P.

Five rabbits were each injected with 1 ml. (500 μc.) of ^{131}I-labelled P.V.P. At the same time 3 of these rabbits were each infected with 50 metacercariae. The first blood sample was removed 24 hours after injection and subsequent samples taken every other day for a total period of 17 days.

The study was continued by repeating the above procedure in two further groups of 5 rabbits (3 infected and 2 controls). In the first of these, the infected animals had each been given 50 metacercariae 17 days previously, while those of the second group had been similarly infected 39 days previously.

(C) Simultaneous Measurement of Intestinal Red Cell and Plasma Loss at Different Stages of Infection - Use of ^{51}Cr-labelled Red Cells and ^{95}Nb-labelled Albumin

(i) Labelling of Albumin with ^{95}Nb

A measured amount of ^{95}Nb-labelled albumin, prepared as previously described and containing about 50 μc ^{95}Nb was injected into the marginal ear vein of each of the experimental animals.

(ii) Labelling of Red Cells with ^{51}Cr

Blood samples taken from each of the experimental animals were incubated with ^{51}Cr (200 μc/ml. packed red cells), washed until free of unbound isotope and prepared for injection as previously described.

(iii) Experimental Procedure

(a) 6 - 8 Weeks Post-infection

Three rabbits which had each been infected with 50 metacercariae 40 days previously and 3 control rabbits were each injected with ^{51}Cr-labelled red cells and ^{95}Nb-labelled albumin at the same time. The first blood sample was withdrawn 5 minutes after injection and further samples were taken daily for 16 days.

(b) 18 - 19 Weeks Post-infection

The infected rabbits used in the above experiment were again each injected with labelled suspensions of albumin and red cells. Four control rabbits were similarly studied. The first blood sample was taken 5 minutes after injection, and subsequent samples removed once daily for 10 days.

(iv) Collection of Urine and Faeces and Measurement of Radioactivity

In all experiments, urine and faeces were collected at regular 24-hour intervals following injection of labelled materials and samples prepared for radioactivity determinations as previously described. One ml. samples of blood and plasma were diluted to 5 ml. for counting and suitable aliquots of standard solutions of labelled preparations assayed at regular intervals. Corrections for radioactive decay and separation of isotopic mixtures were based on the activities of these solutions.

(v) Construction of Disappearance Curves

The count rate of each plasma and blood sample, corrected for radioactive decay was expressed as a % of the 5 minute post-injection sample and a semi-log plot made of activity against time. By use of the venous haematocrit determination on each sample, the radio-activity per ml. of red cells was calculated.

(vi) Analysis of Faecal and Urinary Excretion of Isotopes

Faecal "clearances" of all isotopes were calculated for each 24-hour collection period following injection of each of the labelled materials as previously described. Urinary radioactivity was expressed

as a % of that injected.

RESULTS

In this section of the thesis only tables of mean values of the various parameters of protein and red cell metabolism will be used in the text. Daily "clearances" of plasma and red cells and plasma protein pool sizes, distribution ratios and catabolic rates, together with plasma and red cell disappearance curves are shown in Appendix D.

(A) PHASE 1:- UP TO 5 WEEKS POST-INFECTION

(i) Albumin Distribution and Metabolism

At the beginning of this study, no difference was apparent in the blood composition of the two groups of rabbits (Appendix D). Albumin pool sizes and distribution ratios were also very similar in all animals (Table 28). Catabolism of the labelled albumin and faecal excretion of isotope were not increased in the infected animals during the experimental period.

(ii) Turnover of Labelled Red Cells

The only marked difference between the infected and control animals during the first five weeks was a significant reduction ($P < 0.05$) in the survival of the labelled cells in the circulation of the former group (Table 29). This could not however have resulted from excessive haemolysis since 37.2% (S.D. 8.3) of the injected radioactivity was excreted in the urine of these rabbits compared to 42.9% (S.D. 7.6) in the controls.

Faecal excretion of isotope was not increased during this period (Table 29), indicating that loss of labelled cells into the gut was not

^{125}I Labelled Albumin/ ^{51}Cr Labelled Erythrocytes Experiments in Rabbits Following Infection with F. hepatica

TABLE 28

^{125}I LABELLED ALBUMIN - EXPERIMENTAL RESULTS (MEAN VALUES)

PHASE 1:- DAYS 0 - 38 POST - INFECTION

	INFECTED	CONTROL
Plasma Volume(ml/kg)	36.5	37.7
CA(gm/kg)	1.16	1.13
TA(Sterling,gm/kg)	3.88	3.78
EA(Sterling,gm/kg)	2.73	2.60
TA(Campbell),gm/kg)	3.48	3.41
EA(Campbell,gm/kg)	2.33	2.23
EA/CA(Sterling)	2.39	2.21
EA/CA(Campbell)	2.09	1.89
EA/TA(Sterling)	0.70	0.69
EA/TA(Campbell)	0.67	0.65
$T_{\frac{1}{2}}$ (Hours)	169	164
Equil. Time (Hours)	43	44
F(CA) (Catabolic Rate - Campbell)	0.238	0.217
F(TA)	0.044	0.041
Plasma "Clearance"(ml/24 hours)	0.51	0.58
Absolute Amount of Albumin Catabolised (gm/kg/24 hours)	0.272	0.257

^{125}I Labelled Albumin/ ^{51}Cr Labelled Erythrocytes Experiments in Rabbits Following Infection with F. hepatica

TABLE 29

^{51}Cr LABELLED ERYTHROCYTES - EXPERIMENTAL RESULTS (MEAN VALUES)

PHASE 1:- DAYS 0 - 38 POST - INFECTION

	INFECTED	CONTROL
Blood Volume(ml/kg)	54.9	59.9
Circ. R.B.C. Vol. (ml/kg)	20.2	22.2
$T_{\frac{1}{2}}$ Whole Blood (Hours)	274	387
$T_{\frac{1}{2}}$ Red Cells (Hours)	282	387
Faecal "Clearance"(ml/day)		
Whole Blood	0.16	0.16
Red Cells	0.05	0.06

responsible. It also seems unlikely that haemodilution played any part in the more rapid removal of labelled cells since the "apparent half-life" of the labelled albumin was virtually identical in the infected and control rabbits (Table 28).

(B) A COMPARISON OF ALBUMIN AND RED CELL TURNOVER BETWEEN 5 - 10 WEEKS AND 18 - 20 WEEKS POST-INFECTION

(i) Blood Composition of Normal and Fluke-infected Rabbits

By 35 days post-infection, several differences were apparent in the blood composition of infected and control rabbits (Appendix D). Although the average venous haematocrit of both groups was identical (39%), one of the infected rabbits (rabbit No. 307) was already quite anaemic (P.C.V. 34%). This animal died 7 weeks later and was therefore not included in statistical analysis. Total protein levels were also very similar in all animals (6.27 gms. % S.D. 0.29 in the controls and 6.84 gms. % S.D. 0.55 in the infected). However a significantly decreased albumin/globulin ratio ($P < 0.002$) was found in the infected rabbits remained significantly less than those of the controls.

(ii) Albumin Turnover Studies

(a) Albumin Pool Sizes and Distribution Ratios

No significant difference was apparent between infected and control rabbits either at 35 days or at 18 weeks post-infection in the size of the albumin pools (Table 30). Distribution ratios were likewise very similar in the two groups (Table 30).

^{125}I-labelled Albumin / ^{51}Cr-labelled Erythrocytes Experiments in Rabbits Following Infection with F. hepatica

TABLE 30

^{125}I-LABELLED ALBUMIN - EXPERIMENTAL RESULTS (MEAN VALUES)

	PHASE 2: DAY 35 - 70 POST-INFECTION					PHASE 3: DAY 126 - 140 POST-INFECTION					
	Infected	S.D.	Controls	S.D.	P	Infected	S.D.	Controls	S.D.	P	P_{2-3}
Plasma Volume (ml/kg)	38.94	3.34	38.53	3.67	N.S.	37.20	4.27	35.50	5.01	N.S.	N.S.
CA (gm/Kg)	1.02	0.12	1.23	0.06	N.S.	0.97	0.13	1.09	0.14	N.S.	N.S.
TA (Sterling, gm/Kg)	2.93	0.35	3.06	0.30	N.S.	3.08	0.70	2.94	0.40	N.S.	N.S.
EA (Sterling, gm/Kg)	1.91	0.28	1.83	0.33	N.S.	2.11	0.59	1.85	0.45	N.S.	N.S.
TA (Campbell, gm/Kg)	2.97	0.38	2.93	0.25	N.S.	2.72	0.55	2.48	0.27	N.S.	N.S.
EA (Campbell, gm/Kg)	1.96	0.32	1.70	0.30	N.S.	1.75	0.43	1.39	0.31	N.S.	N.S.
EA/CA (Sterling)	1.89	0.28	1.49	0.31	N.S.	2.16	0.42	1.73	0.39	N.S.	N.S.
EA/CA (Campbell)	1.93	0.30	1.39	0.29	N.S.	1.79	0.24	1.30	0.42	N.S.	N.S.
EA/TA (Sterling)	0.65	0.03	0.59	0.05	N.S.	0.68	0.04	0.62	0.08	N.S.	N.S.
EA/TA (Campbell)	0.65	0.04	0.58	0.06	N.S.	0.64	0.03	0.55	0.08	N.S.	N.S.
$T\frac{1}{2}$ (hrs)	126.2	6.50	178.3	20.21	<0.002	97.5	18.21	171.7	12.5	<0.002	<0.02
Equil. Time (hrs)	54.4	6.98	53.0	3.61	N.S.	39.8	8.18	50.3	14.15	N.S.	<0.05
F(CA) (K-Campbell)	0.304	0.030	0.175	0.037	<0.002	0.440	0.088	0.225	0.081	<0.05	<0.02
K Matthews	0.331	0.040	0.212	0.028	<0.01	0.461	0.099	0.238	0.066	<0.05	<0.02
F (TA)	0.043	0.010	0.040	-	N.S.	0.140	0.010	0.101	0.017	<0.02	<0.001
Plasma Clearance (ml/24hrs)	1.58	0.50	0.56	0.18	<0.02	1.54	0.948	0.323	0.047	<0.05	N.S.
Absolute Amount Albumin Catabolised (gm/Kg/24hrs)	0.31	0.04	0.254	0.04	N.S.	0.43	0.117	0.238	0.059	<0.05	N.S.

P = Significance of Difference Between Infected and Controls

P_{2-3} = Significance of Difference Between Phase 2 and Phase 3. Infected Animals.

(b) Catabolism of Labelled Albumin

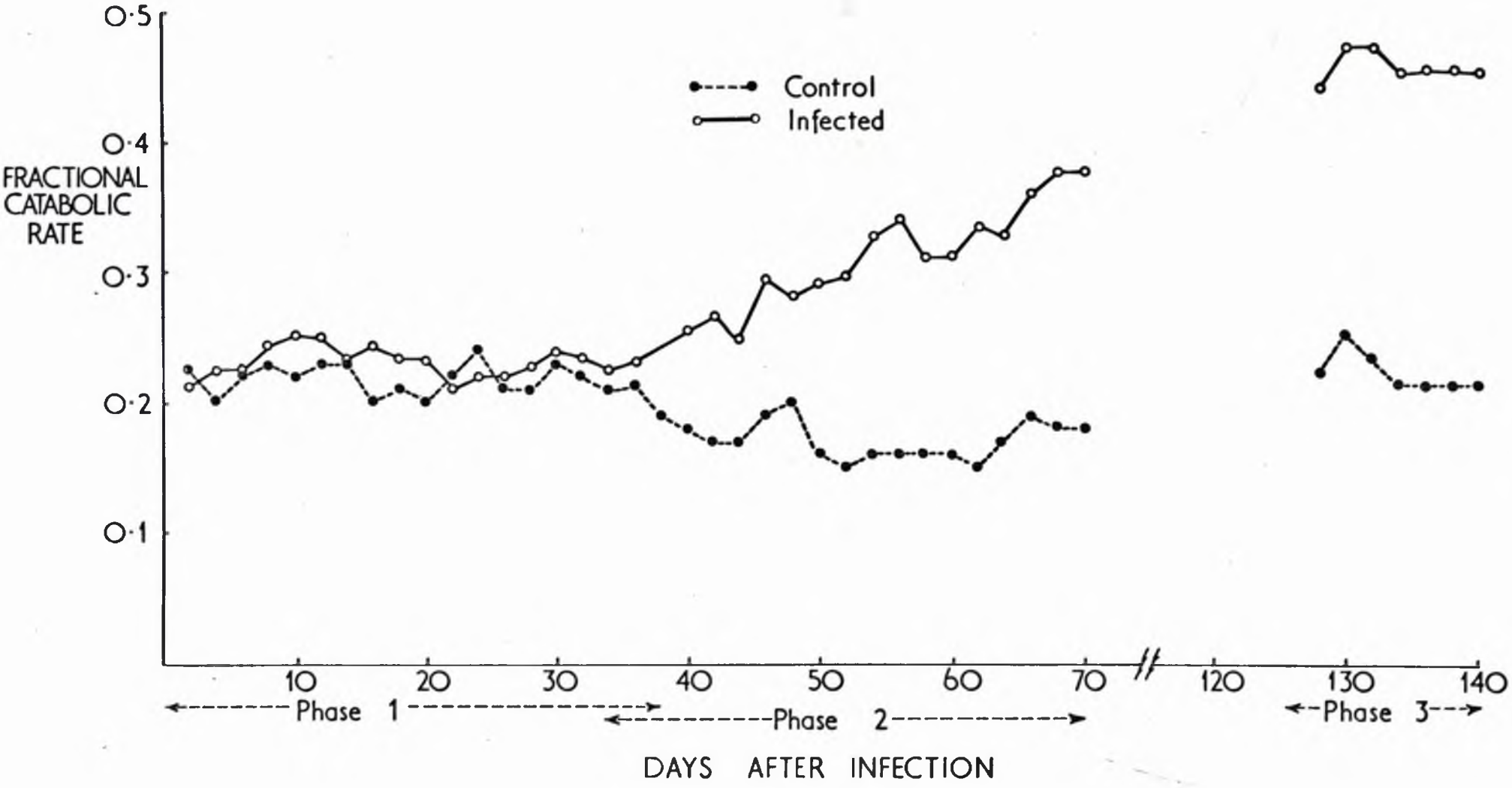
It is clear from the results in Table 30, that the albumin degradation rate between days 35 and 70 post-infection was considerably greater in the fluke-infected animals. This was shown by the shortened "apparent half-life" and also by the elevated values for the fractional catabolic rate calculated both by graphic resolution of the plasma disappearance curves and by analysis of the radioactivity excreted.

The onset and development of this hypercatabolism is illustrated in Fig. 22. Whereas no significant difference was found in albumin degradation between the infected and control animals over about the first 6 weeks after infection, from this time onwards, the fractional catabolic rate of the labelled albumin in the infected group (calculated by the method of Campbell et al 1956) increased progressively until at 10 weeks post-infection 36% of the intravascular pool was catabolised daily. During this period, the fractional catabolic rate in the controls, apart from some minor fluctuations, remained at about 20% per day.

In order to establish whether catabolic rates increased further after 10 weeks, these same rabbits were injected 3 weeks later with a labelled albumin preparation and turnover studies continued for a further period of 14 days. In the controls albumin degradation expressed as an "apparent half-life" and as a fractional catabolic rate was almost identical to that obtained during the previous experiment. However the rate of albumin degradation had increased very significantly in the infected rabbits between 10 and 18 weeks post-infection ($P < 0.02$). For example,

FIGURE 22

^{125}I LABELLED ALBUMIN and ^{51}Cr LABELLED ERYTHROCYTES FRACTIONAL CATABOLIC RATE of ALBUMIN FOLLOWING INFECTION of RABBITS with F. HEPATICA



the mean daily fractional catabolic rate had increased to 44% - a 20% increase over the 10 week post-infection figure. In addition the "apparent half-life" of the labelled albumin was reduced to about 50% of the control value (Table 30).

Although significant differences between infected and control animals in both the fraction of the total body pool F(TA) and in the absolute amount of albumin catabolised per day were not apparent over the 5 - 10 week period, these were very much greater in the infected rabbits between 18 and 20 weeks than in the controls (Table 30).

(c) Faecal Excretion of Isotope

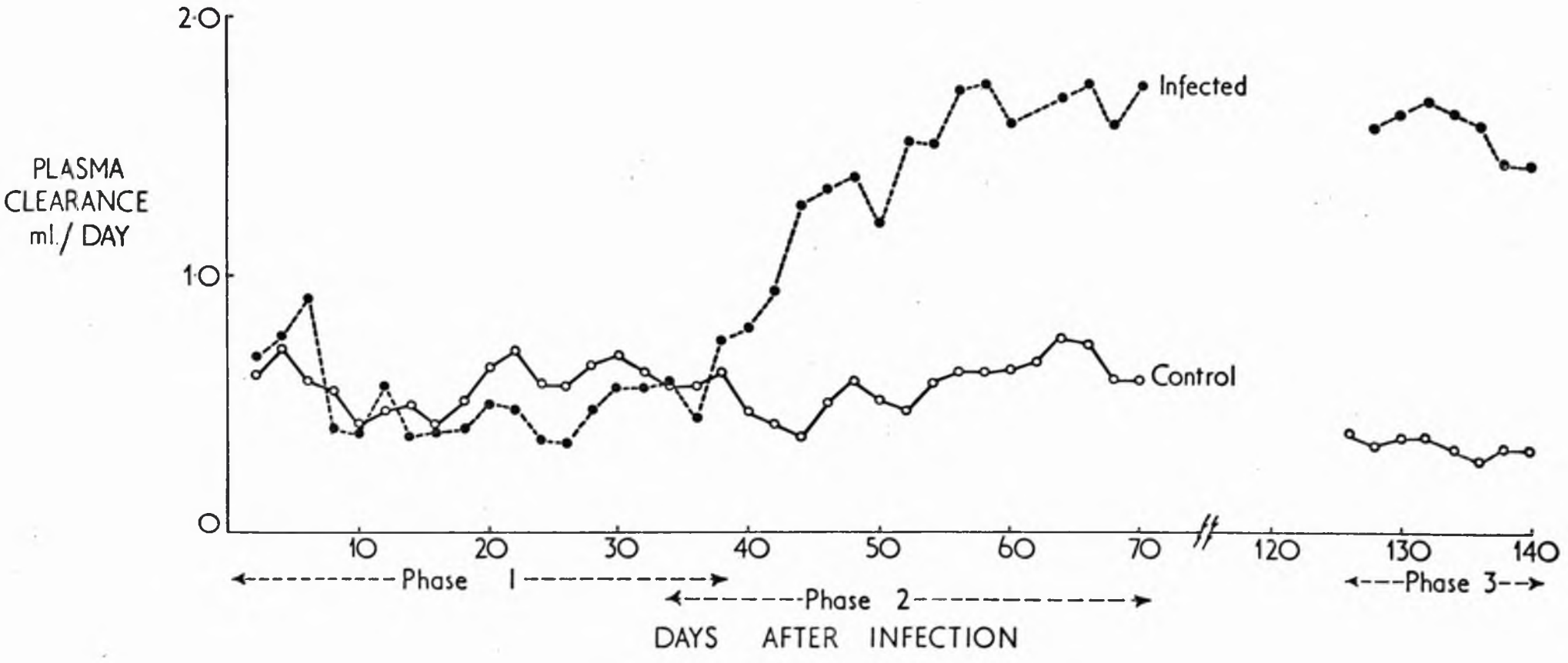
Daily faecal "clearances" of plasma were calculated throughout the above experiments and plotted against time after injection (Fig. 23). A progressive increase in "clearance" was associated with the infected rabbits, commencing at about 38 - 42 days post-infection. It is noteworthy that no further increase occurred between 18 and 20 weeks post-infection, although the cumulative faecal activity of the infected rabbits expressed as a percentage of that injected over this period was 3.5% (S.D. 1.6) compared to 1.5% (S.D. 0.4) in the controls.

(d) Estimation of Macromolecular Gut Loss - Faecal Excretion of ^{131}I-labelled P.V.P.

Faecal "clearances" of plasma based on studies with radioiodinated proteins are of course a gross underestimate of the amount of plasma actually leaking into the gut each day because of the substantial breakdown and reabsorption of label that occurs. Thus, in order to obtain a more

FIGURE 23

^{125}I-LABELLED ALBUMIN and ^{51}Cr-LABELLED ERYTHROCYTES MEAN DAILY PLASMA CLEARANCES in RABBITS FOLLOWING INFECTION with F. HEPATICA



quantitative estimate of enteric plasma loss, movement of plasma macromolecules into the gut was directly measured by injecting ^{131}I-labelled P.V.P. into groups of infected and control rabbits and following the plasma levels and faecal excretion of isotope as previously described. The average daily "clearances" plotted against time after infection are shown in Fig. 24.

After about 6 weeks, the mean "clearance" of the infected rabbits increased progressively, until at 10 weeks, it was about six times greater than that of the controls. The similarity in the time of onset of albumin hypercatabolism and increased movement of plasma macromolecules into the gut of infected rabbits is very striking.

Conclusions

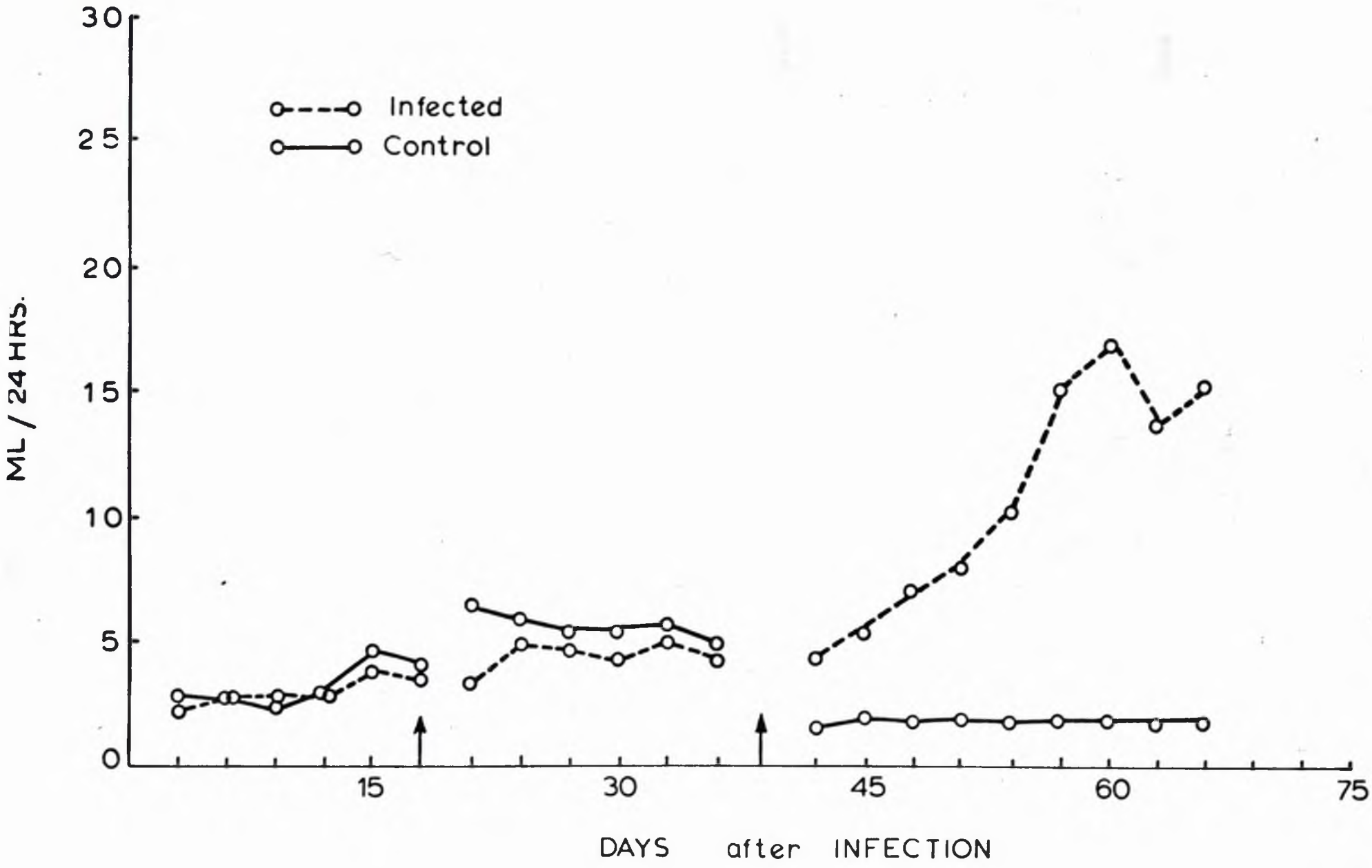
The results of these experiments show that only after about 6 weeks post-infection i.e. when the flukes are becoming established within the bileducts is labelled albumin degraded at a significantly faster rate than in normal animals.

Coinciding with the onset and progressive development of this hypercatabolism was a striking increase in the radioactivity excreted in the faeces of the infected rabbits, indicating that a direct loss of albumin into the gastrointestinal tract was responsible. Further evidence in favour of this theory was obtained from the studies on the faecal excretion of labelled P.V.P.

It is noteworthy that the albumin degradation rate continued to increase even after the flukes had become established within the bile

FIGURE 24

DAILY FAECAL CLEARANCE of ^{131}I - LABELLED P.V.P. in RABBITS FOLLOWING INFECTION with F. HEPATICA



ducts, and although no studies were carried out between 10 and 18 weeks post-infection, because of the constancy of the fractional catabolic rate during the 18 - 20 week period, it would appear that in the rabbit, at some time between 10 and 20 weeks post-infection, degradation reached a "steady" maximum value.

Although no significant increase in faecal "clearance" of isotope was noted to explain the greater catabolic rate at 18 weeks relative to 10 weeks post-infection, it should be emphasised that radioactivity excreted in the faeces following injection of radioiodinated proteins is derived from secretion of radioiodide into the gut, and although the appearance of excessive amounts of the isotope in the faeces is a good qualitative test of protein loss into the digestive tract, it cannot be used to quantitatie such losses because of reabsorption. Thus, in order to explain the further increase in albumin catabolism at 18 weeks post-infection, it is necessary to use a label which is not reabsorbed on entry into the gut. This aspect of the disease was studied using ^{95}Nb-labelled albumin.

(iii) Turnover of ^{51}Cr-labelled Red Cells

The onset and development of the anaemia associated with F. hepatica infections was simultaneously studied by analysis of the blood levels and faecal and urinary excretion of isotope following injection of ^{51}Cr-labelled red cells. Red cell turnover measurements were carried out between 5 and 10 weeks post-infection and repeated between 18 and 20 weeks.

(a) Red Cell and Blood Volumes

No difference was apparent in either the blood or circulating red cell volumes of infected and control animals at 35 days post-infection (Table 31), and even rabbit No. 307, which was already quite anaemic at this time, showed no reduction in red cell volume (Appendix D). However, by 16 weeks post-infection, the red cell volumes of the infected rabbits were significantly lower than those of the controls (Table 31).

(b) Persistence of Labelled Cells

Over the 5 - 10 week post-infection period, a striking increase in the rate of disappearance of the labelled cells from the circulation of the infected relative to the control animals was apparent (Table 31). It should be noted that the half-life value attributed to each of the infected rabbits (Appendix D), was calculated from the blood radio-activity over the entire experimental period. These do not however give any indication of the precipitous decline in blood activity which occurred in each of these rabbits between 48 and 55 days post-infection. Red cell activity curves obtained for one of the normal and one of the fluke-infected rabbits illustrate this point (Fig. 25). It is also noteworthy that although the most rapid loss of labelled cells from the circulation of this rabbit occurred after about 55 days, the survival of these cells was still considerably reduced between 5 and 8 weeks post-infection relative to those of the control. Although differences were apparent in the rate and time at which the sudden decline in red cell radioactivity occurred, the same general pattern of red cell survival

^{125}I-labelled Albumin/ ^{51}Cr-labelled Erythrocytes Experiments in Rabbits Following Infection with F. hepatica

TABLE 31

^{51}Cr-LABELLED ERYTHROCYTES - EXPERIMENTAL RESULTS(MEAN VALUES)

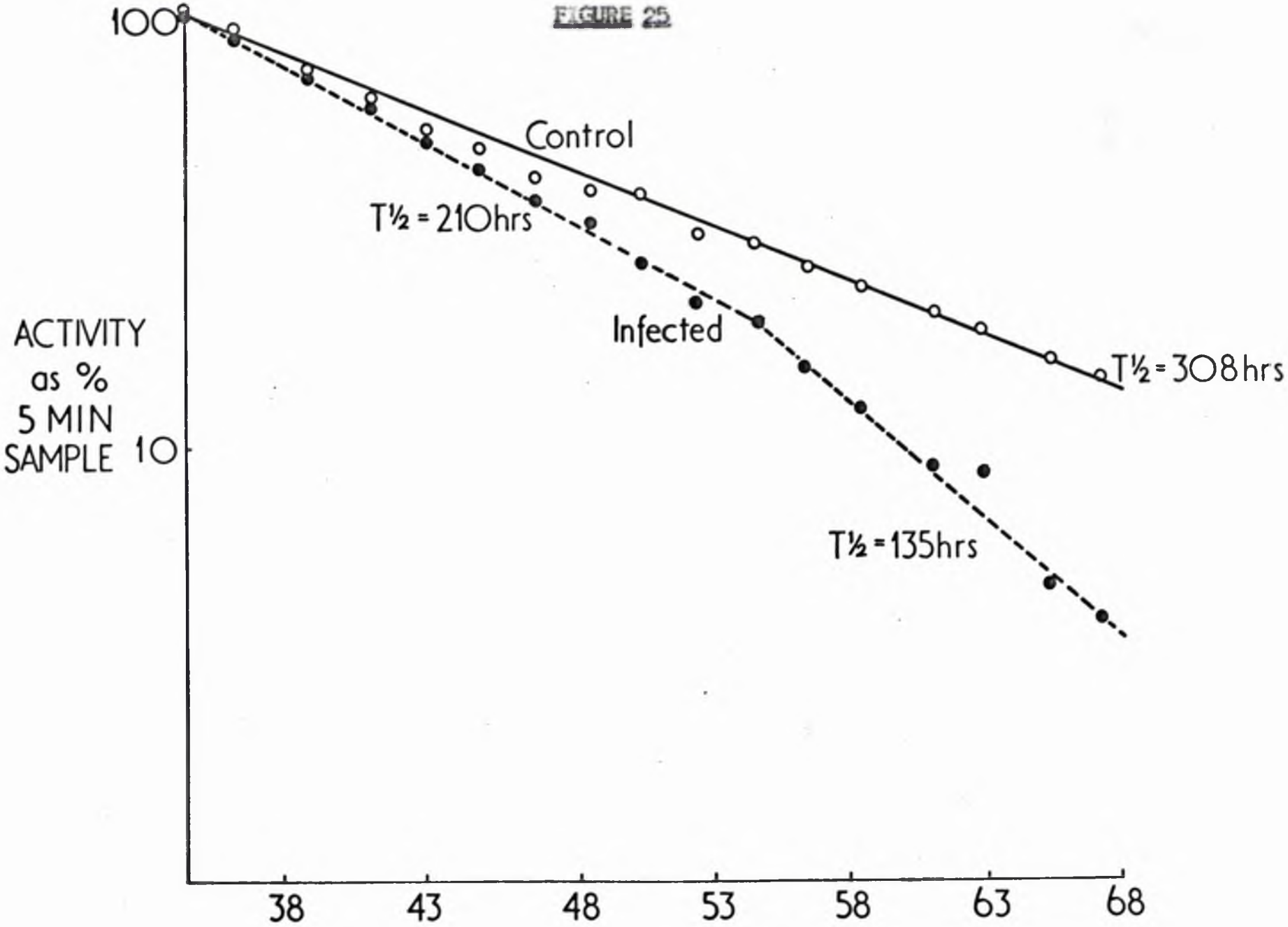
PHASES 2 and 3

RABBIT	PHASE2:- DAYS 35-70 POST-INFECTION					PHASE 3:- DAYS 126-140 POST-INFECTION				
	Blood Volume (ml/kg)	Circ.Vol. R.B.C. (ml/kg)	R.B.C. $T_{\frac{1}{2}}$ (Hours)	Faecal"Clearance" Blood (ml/24hr)	Faecal"Clearance" R.B.C. (ml/24hr)	Blood Volume (ml/kg)	Circ.Vol. R.B.C. (ml/kg)	R.B.C. $T_{\frac{1}{2}}$ (Hours)	Faecal"Clearance" Blood (ml/24hr)	Faecal"Clearance" R.B.C. (ml/24hr)
CONTROL	52.6	14.1	308	0.08	0.03	49.0	13.1	365	0.05	0.02
S.D.	6.5	3.1	7.6	0.04	0.01	4.9	0.7	67	0.01	-
$P_{2/3}$	N.S.	N.S.	N.S.	N.S.	N.S.					
INFECTED	53.4	14.7	163	6.2	1.8	46.7	9.5	114	12.4	2.6
S.D.	4.7	1.7	36	4.1	1.1	5.3	1.9	51	6.7	0.8
P	N.S.	N.S.	<0.001	<0.05	<0.05	N.S.	<0.05	<0.01	<0.05	<0.01
$P_{2/3}$	N.S.	<0.02	N.S.	<0.05	<0.05					

$P_{2/3}$ - Significance of Difference Between Phases 2 and 3 in Infected and Control Animals.

Cr-LABELLED ERYTHROCYTES - R.B.C. ACTIVITY FOLLOWING INFECTION
with F. HEPATICA PHASE 2 DAY 35-70 POST-INFECTION

FIGURE 25



was associated with each of the infected rabbits between 5 and 10 weeks post-infection.

In all but one of the infected rabbits (rabbit No. 304, from which 10 flukes were recovered at autopsy), a 50% reduction was found in the half-life of the labelled cells at 18 weeks post-infection, relative to the 5 - 10 week value (Appendix D). It was clearly only because of the small number of animals used in the experiments, and the degree of scatter, that these half-life differences were not statistically significant.

An excellent correlation was obtained between the half-life of the labelled cells at 18 weeks post-infection and the number of flukes recovered at autopsy (Appendix D).

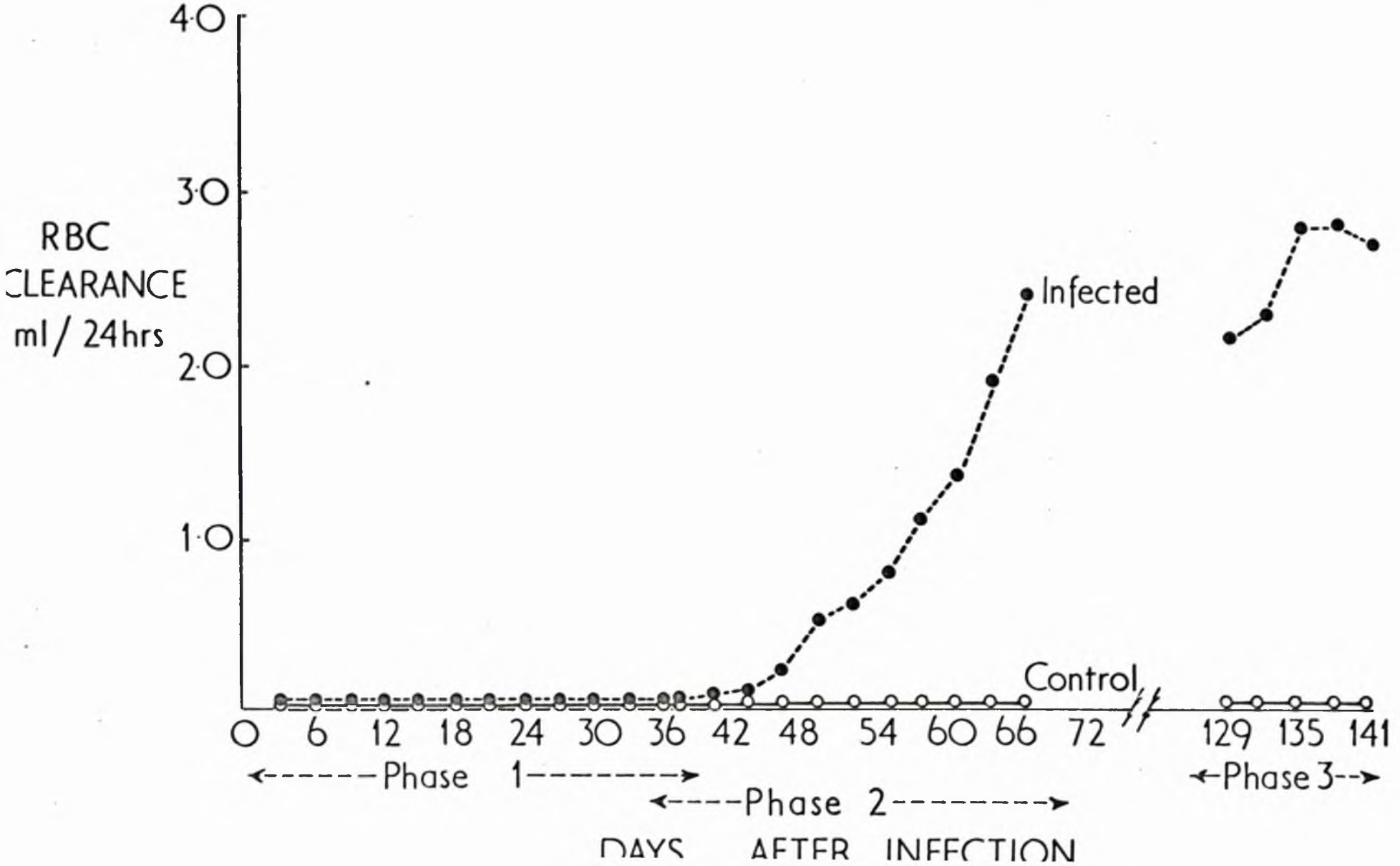
(c) Faecal Excretion of ^{51}Cr

Daily faecal "clearances" of whole blood and red cells were calculated for each rabbit and the mean red cell values obtained for both groups plotted against time after infection (Fig. 26). Between 6 and 10 weeks the average clearance of the infected rabbits increased from less than 0.05 ml. to over 2 ml./day while that of the controls remained at the lower figure. These "clearances" are shown in Table 31, the value for the infected rabbits being the mean figures obtained between 8 and 10 weeks post-infection.

Whereas the earliest marked increase in "clearance" was noted in rabbit No. 307 at about 40 days, those of rabbit No. 304 were not greatly elevated until 46 days after infection. Thus although some

FIGURE 26

^{125}I-LABELLED ALBUMIN and ^{51}Cr-LABELLED ERYTHROCYTES MEAN DAILY RED CELL CLEARANCE in RABBITS FOLLOWING INFECTION with F. HEPATICA



variation was found in the onset of increased faecal excretion of isotope, this was not great.

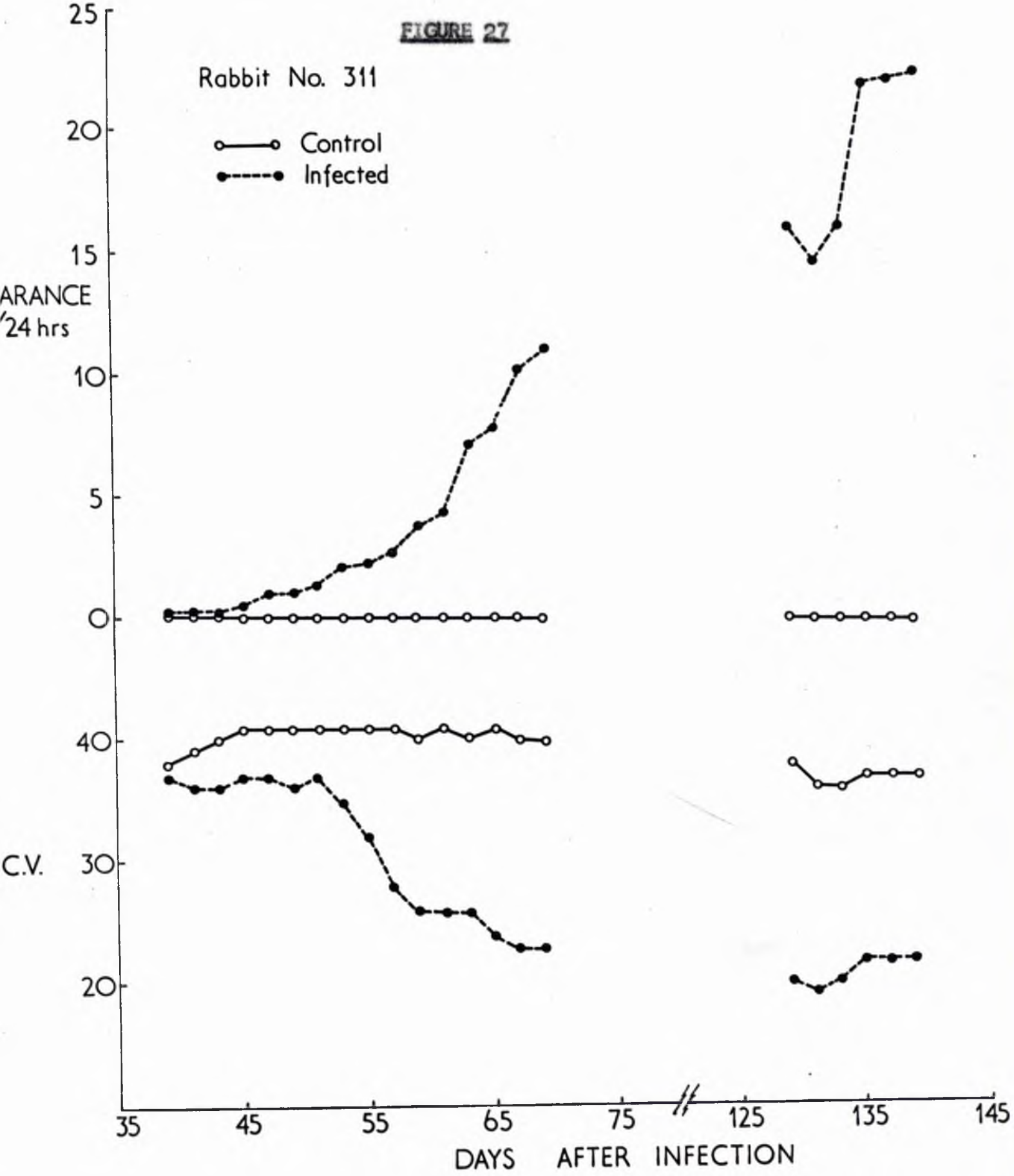
It was also very apparent that the rate at which the faecal "clearances" increased was closely related to that at which the P.C.V. of the infected animals decreased. There was in addition a close correlation between both of these factors and the number of flukes recovered at autopsy. For example, the "clearance" of whole blood in rabbits Nos. 310 and 311, from which 26 and 27 flukes were respectively recovered increased to about 12 ml./day between 5 and 10 weeks post-infection. Over the same period, the P.C.V. of both animals fell from 38% to 22%. However, the P.C.V. of rabbit No. 304 from which only 10 flukes were recovered was reduced from 37% to 34%, while the average "clearance" increased progressively to 3ml./day. The changes in P.C.V. and blood "clearance" with time are shown for each rabbit in Appendix D. It is noteworthy that although the average daily red cell "clearance" of the infected rabbits at 18 - 20 weeks post-infection (2.6 ml/day S.D. 0.8) was significantly greater than that obtained between 8 and 10 weeks (1.8 ml./day S.D. 1.1), P.C.V. values of all infected rabbits were however only slightly lower at 18 weeks than at 10 weeks post-infection (Fig. 27).

Conclusions

No clear-cut explanation can be offered to account for slight but significant reduction in survival of ^{51}Cr-labelled red cells in the fluke-

^{125}I-LABELLED ALBUMIN and ^{51}Cr-LABELLED ERYTHROCYTES P.C.V. CHANGES and BLOOD CLEARANCES FOLLOWING INFECTION with F. HEPATICA

FIGURE 27



infected rabbits during the migratory phase of the disease (Table 29). Since faecal and urinary excretion of isotope was no greater than in the controls, neither loss of labelled cells into the gut nor excessive haemolysis could explain this phenomenon. Urquhart (1955) described the pathological lesions associated with migration of the young flukes through the liver tissue to the bile ducts of the rabbit. He noted that the space left in the wake of the flukes as it burrowed through the parenchyma was filled with cell debris and red cells. It therefore seems possible that the labelled erythrocytes became "trapped" in these spaces within the liver tissue, thereby reducing their half-life without increasing the excretion of isotope. Since neither the P.C.V. nor circulating red cell volume of infected rabbits was reduced before 6 weeks post-infection it is clear that loss of erythrocytes from the circulation by this mechanism was not of any significant importance in the aetiology of the anaemia associated with the disease.

The results of these studies confirm that the primary influence in causing the anaemia was loss of red cells into the gut of the infected rabbits. There were however several interesting features about the onset and increase in severity of the anaemia.

Firstly, increased faecal excretion of isotope was apparent in all the infected rabbits for several days before either the P.C.V. or the half-life of the labelled cells was dramatically reduced. The results of these studies indicate that only after the blood "clearance" reached

about 1.5 - 2 ml. and was maintained at or increased beyond this value for 2 - 3 days did the P.C.V. of the infected rabbits fall significantly. Secondly, the time of onset of the marked anaemia and sudden decline in blood radioactivity were very similar in all rabbits. For example, the half-life of the labelled cells in rabbit No. 310 decreased from 210 hours to 135 hours at about 55 days post-infection (Fig. 25), while between days 54 and 57, the P.C.V. fell from 33% to 28% (Appendix D).

Thirdly, although the average red cell "clearance" increased by about 30% between 8 and 18 weeks post-infection, the P.C.V. of the infected animals remained fairly steady (although at a slightly lower value) between 18 and 20 weeks. It would therefore appear that by this time, the rabbits' erythropoietic system had become adapted to the blood loss.

(C) A STUDY OF THE RELATIONSHIP BETWEEN PLASMA AND RED CELL LOSS AT DIFFERENT STAGES OF INFECTION - SIMULTANEOUS USE OF ^{95}Nb-LABELLED ALBUMIN AND ^{51}Cr-LABELLED RED CELLS

While the above results show that both the anaemia and increased albumin degradation only become apparent when the flukes enter the bile ducts they give no information about the relationship between plasma and red cell loss at different stages of infection. Although it was previously shown (Section 3) that the passage of plasma proteins into the gut of rabbits with long-standing infections was greater than could be accounted for by a loss of whole blood, no comparison was made with the situation existing at about the time the flukes enter the biliary system.

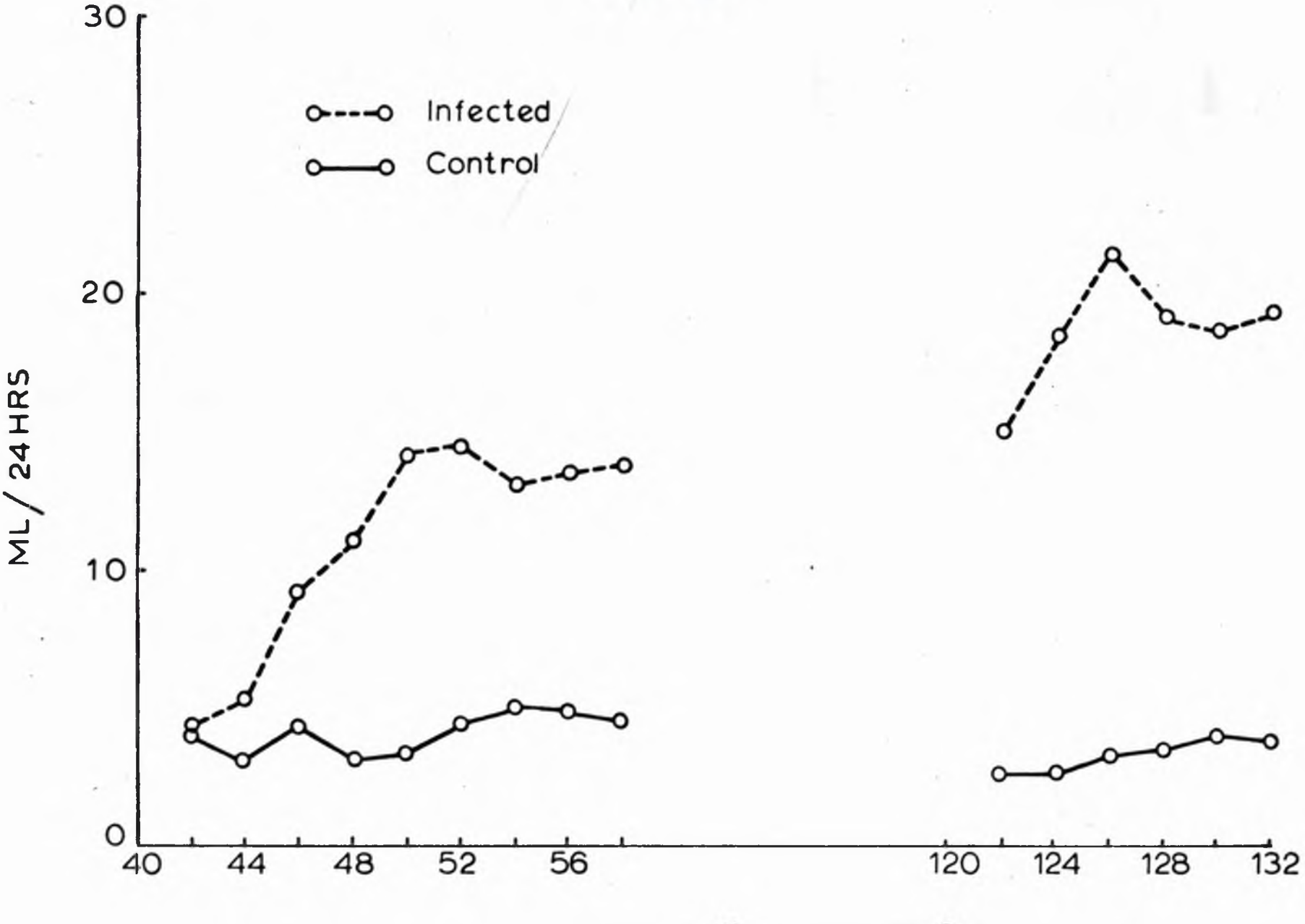
Such a comparison may perhaps throw some light on the aetiology of the further increase in albumin degradation which was previously noted between 10 and 18 weeks post-infection.

This aspect of the disease was studied by injecting ^{95}Nb-labelled albumin and ^{51}Cr-labelled red cells into 3 normal rabbits and 3 rabbits which had each been infected with 30 metacercariae 40 days previously. The blood level and faecal and urinary output of both isotopes were measured over the following 16 days and daily "clearances" of plasma, blood and red cells calculated. These measurements were repeated 10 weeks later (18 weeks post-infection) following a further injection of labelled albumin and erythrocytes. Three different control rabbits (together with rabbit No. 56) were used for this study.

Daily "clearances" of plasma and red cells plotted against time after infection are shown in Figs. 28 and 29 respectively. A very striking increase in both plasma and red cell "clearance" was associated with the infected rabbits, commencing as before at about 6 weeks after infection and thereafter increasing rapidly over the entire experimental period. By 18 weeks post-infection these "clearances" had increased to values significantly greater than those obtained between 6 and 8 weeks. The mean "clearances" of both experimental periods are shown in Table 32. Even between 6 and 8 weeks post-infection there appeared to be a preferential leak of plasma, and if the "background" leak as manifested by the plasma "clearance" of the control animals is subtracted from the mean infected "clearances" obtained during both periods, it is clear that

^{95}Nb-LABELLED ALBUMIN and ^{51}Cr-LABELLED ERYTHROCYTES
DAILY PLASMA CLEARANCES in RABBITS FOLLOWING INFECTION with F. HEPATICA

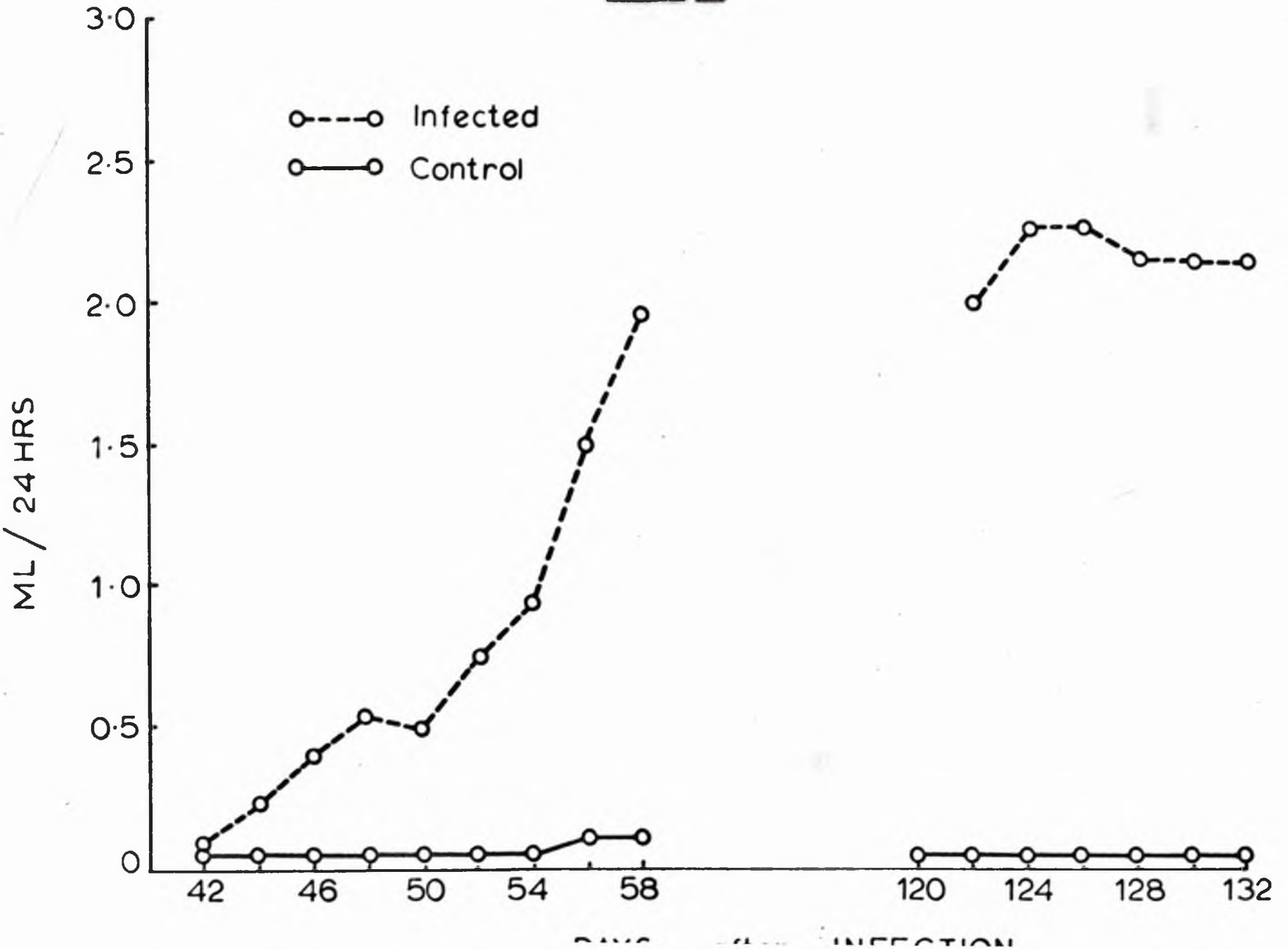
FIGURE 28



^{95}Nb-LABELLED ALBUMIN and ^{51}Cr-LABELLED ERYTHROCYTES

DAILY RED CELL CLEARANCES in RABBITS FOLLOWING INFECTION with F. HEPATICA

FIGURE 29



^{95}Nb-labelled Albumin/ ^{51}Cr-labelled R.B.C. Experiments in Rabbits Following Infection with F. hepatica

TABLE 32

MEAN DAILY PLASMA, BLOOD AND RED CELL "CLEARANCES" (ML.)

	Rabbit No.	6 - 8 Weeks Post-infection			18 - 19 Weeks Post-infection			Flukes Recovered
		Plasma	Blood	R.B.C.	Plasma	Blood	R.B.C.	
INFECTED	095	13.2	3.5	1.05	25.8	13.4	3.9	27
	088	8.5	2.8	0.94	16.6	11.9	3.1	23
	63	7.6	0.7	0.28	23.7	5.7	1.8	12
	Mean	9.8	2.3	0.76	22.0	10.3	2.9	21
	S.D.	3.0	1.5	0.42	4.8	4.1	1.1	
CONTROL	48	3.4	0.10	0.04	-	-	-	
	103	4.5	0.20	0.07	-	-	-	
	56	4.5	0.21	0.08	4.1	0.13	0.04	
	177	-	-	-	3.1	0.08	0.03	
	197	-	-	-	3.3	0.13	0.05	
	199	-	-	-	3.7	0.16	0.02	
	Mean	4.1	0.17	0.06	3.6	0.13	0.04	
	S.D.	0.6	0.06	0.02	0.4	0.03	0.01	
	P	<0.05	<0.05	<0.05	<0.001	<0.01	<0.01	

about twice as much plasma was lost into the gut than would be expected from a leak of whole blood.

A good correlation was again found between the magnitude of the "clearances" and the number of flukes recovered at autopsy (Table 32).

Conclusions

The further increase in plasma "clearance" of each infected rabbit between about 8 and 18 weeks post-infection provides a rational explanation for the higher degradation rate of albumin in rabbits with longer-standing infections.

DISCUSSION

It is clear from the above results that both albumin and red cell turnover rates were within normal limits during the migratory phase of the disease, and that only at about the time the flukes entered the bile ducts did anaemia and albumin hypercatabolism become apparent. Thereafter a progressive increase in the severity of the anaemia and in the rate of degradation of labelled albumin occurred in the infected rabbits. Both the onset and development of the disturbances in plasma protein and red cell metabolism were directly attributable to increased intestinal loss of these blood constituents.

Although serum albumin levels of the infected rabbits were slightly reduced relative to those of the controls at 5 and again at 18 weeks post-infection, only very minor differences in albumin pool sizes and distribution ratios were apparent, despite an increase of about 150% in the rate of albumin degradation over this period. It is apparent that albumin synthesis, although not directly measureable with radioiodinated plasma proteins, must also have been increased during this time and at a rate closely parallel to that of catabolism.

It is noteworthy that whereas rabbits infected with either 100 (Section 1) or 50 metacercariae degraded albumin at very similar rates (0.45 and 0.43 gm/kg/day respectively), only in the former group did a significant reduction in the size of the total albumin pool occur (Table 15), suggesting that although albumin synthesis was substantially increased in both groups relative to the controls, rabbits infected with 100 metacercariae

were unable to increase albumin production to the same extent as those which had received only 50 metacercariae.

Although no evidence of impaired albumin synthesis was found during the migratory phase of the disease in this group (i.e. albumin degradation rates and pool sizes of infected and control rabbits were similar at 5 weeks post-infection), the damage caused by migration of larger numbers of immature flukes through the liver could be sufficiently extensive to reduce the ability of these animals to increase albumin production in response to the increased degradation of this protein when the flukes enter the bile ducts. This should not however be taken to indicate that subnormal synthesis played a significant part in the aetiology of the hypoalbuminaemia occurring in these more heavily infected animals - clearly synthesis was greatly increased, but it would appear that relative to those rabbits which were infected with only 50 metacercariae, synthesis was not increased to the same extent.

It would therefore appear that the number of flukes which migrate through the liver probably have some effect on the subsequent ability of this organ to increase its albumin output when the flukes enter the bile ducts, and although liver regeneration in response to damage is obviously of great importance to this end, the extent to which albumin synthesis can be increased is also clearly dependent upon the number of albumin-producing liver cells which escape injury during the migratory phase.

Several interesting points regarding the haemopoietic responses of rabbits to infection with F. hepatica may be made from the studies in this and preceding sections of the thesis.

Since the P.C.V. values of the infected rabbits were maintained for at least 7 days after significant elevations in blood clearance were first noted (Fig. 27 and Appendix D), it would appear that the amount of erythroid marrow existing at that time was capable of increasing red cell output sufficiently to keep pace with this increased loss.

However, whereas over the following 10 days, the progressive increase in "clearance" was accompanied by a rapid decline in the P.C.V. values of most of the infected rabbits, between about 8 and 10 weeks after infection, the rate at which the venous haematocrit decreased was considerably reduced despite a further substantial increase in "clearance" over this period. In addition, by 18 weeks, although the average "clearances" had doubled relative to those obtained between 8 and 10 weeks, P.C.V. values in general fell only by a further 2 - 4%, and during the following 2 weeks, were maintained at this lower level. Clearly, blood loss must have been compensated for to a large extent by a marked increase in erythropoiesis.

It is generally considered that increased red cell and haemoglobin production is brought about by an increase in the mass of erythroid precursors rather than in an increase in the rate of maturation of the individual red blood cells, although the latter cannot be excluded as a contributory factor (Dacie 1954). Determination of the percentage

reticulocytes in the peripheral blood affords one of the best measures of increased erythropoiesis. Urquhart (1955) studied the onset and development of reticulocytosis in fluke-infected rabbits, and noted that by about 60 days after infection, reticulocyte counts had increased from about 1.1% to between 7.5 and 15% and remained elevated throughout the studies which in some cases were continued for a period of over 1 year after infection.

Direct evidence of increased erythropoiesis was obtained from the ferrokinetic studies described in Section 2, in which the rate of red cell production in rabbits which had been infected with 50 metacercariae 3 months perviously was increased by about fourfold. However the fact remains that between 5 and 18 weeks after infection, both the circulating red cell volume and venous haematocrit of each of the infected rabbits used for the studies described in this section fell very dramatically, and it was only after a period of about 10 days, during which time the P.C.V. values of some of these animals had fallen by 30%, that increased red cell production appeared to have "caught up" with the increased loss of cells from the circulation. Thus although the amount of red marrow probably increased as soon as the first flukes entered the bile ducts, its rate of increase was initially not sufficient to keep pace with the continuously increasing loss of cells due to haemorrhage into the gut.

The increased loss or degradation of albumin and rod cells which was associated with the longer established infections was presumably related to the further growth and development of the parasite after

entering the biliary system. Whereas Urquhart (1955) noted that individual flukes which entered the bile ducts at about 5 weeks after infection were roughly 3 mm. in length, those recovered at autopsy (20 weeks) from the rabbits used in the above studies were usually 12 - 19 mm. long. Making the assumption that the adult parasite is haematophagic (and the results reported in this thesis support this theory), it would be expected that its demands for nutrients would increase because of its greater size and increased egg output at 18 compared to 8 weeks after infection.

SUMMARY

(1) Using rabbit albumin trace-labelled with ^{125}I, and simultaneously erythrocytes labelled with ^{51}Cr, the turnover of the blood constituents was studied in rabbits over a 10-week period following infection with 50 metacercariae.

No abnormal leak of either albumin or red cells into the gut of infected animals occurred until about 6 weeks after infection (i.e. at about the time the parasites enter the bile ducts); however from this time onward the infected rabbits showed progressive hypercatabolism and increased gut leak of both albumin and red cells relative to the controls. These losses were followed by a marked reduction in the P.C.V. values of the infected rabbits.

(2) Confirmation of the onset of a significant transfer of plasma macromolecules into the gut of infected rabbits at 6 weeks post-infection was obtained from studies on the faecal excretion of labelled P.V.P.

(3) The extent to which the pathogenic effects of the parasite increase following establishment within the bile ducts was assessed by comparing in the same animals, albumin degradation rates and loss of red cells into the gut at 18 weeks after infection with those measurements 10 weeks previously. Hypercatabolism of albumin and intestinal loss of red cells were much more pronounced in the longer established infection.

(4) The aetiology of this further increase in albumin catabolism and the relationship between plasma and red cell loss was determined by comparing

the faecal "clearances" of plasma and red cells between 6 and 8 weeks with those obtained between 18 and 19 weeks after infection by the simultaneous use of ^{95}Nb-labelled albumin and ^{51}Cr-labelled red cells.

"Clearance" values of both plasma and red cells were significantly greater in the infected rabbits at 18 weeks than at 8 weeks after infection, suggesting that blood loss suffered by the host is determined not only by the number of parasites present within its bile ducts, but also by their size and nutritional requirements.

GENERAL DISCUSSION

From the experiments described in this thesis, it isclear that by the application of radioisotopic techniques to the study of parasitic infections, a great deal of unique information may be obtained enabling not only a more precise assessment of the pathogenic effects of parasites on their hosts, but also a better understanding of the basic physiological responses of the host to the presence of the parasite.

Studies on the distribution and turnover of albumin and IgG immunoglobulin for instance highlight the inadequacy of the more conventional analytical techniques for elucidating the mechanisms responsible for the serum protein changes associated with F. hepatica infections. To understand the aetiology of hypoalbuminaemia or hypergammaglobulinaemia (or an elevated or depressed serum level of any blood constituent), it is necessary to be able to assess the relative importance of subnormal synthesis and increased catabolism or loss, i.e. apart from measuring the concentration and size of the body pool of the constituent concerned, it is important to know the rate at which that constituent is being added to and withdrawn from the pool. This information can only be obtained by the use of isotopically labelled tracer substances. The plasma protein turnover studies described in this thesis showed that although albumin and gammaglobulin were degraded at rates which were roughly twice those of normal rabbits, the intravascular pools of these proteins in fluke-infected animals were not significantly reduced - in fact in the case of IgG this pool was considerably greater in the parasitised group.

Studies involving the use of radioiodinated plasma proteins in addition to providing valuable data on distribution and catabolism, usually enable certain inferences to be drawn about the rates of synthesis of these molecules. The finding that the production of gammaglobulins can be increased to a greater extent than that of albumin is probably explained by the greater number of cells producing these proteins. Thus, whereas gammaglobulin is synthesised by the plasmocytic cell lines of the reticuloendothelial system and the number of these cells may increase manyfold in response to antigenic stimulation, the fact that hyperalbuminaemia isn't known fits in well with the hypothesis that the rate of albumin synthesis in the liver depends on the concentration of albumin or the concentration gradient in the liver capillaries. Accordingly, a low level of albumin results in an increased mitotic activity in the liver and an increased rate of albumin synthesis.

Although serum protein changes similar to those reported in chronic F. hepatica infections have been described in many parasitic diseases, few studies on the turnover of the proteins involved have been carried out, and therefore where such changes occur, the precise mechanisms responsible must at the present time be purely conjectural since it is not known whether they are due to subnormal synthesis, increased catabolism or both.

With the development of suitable labels for the detection of abnormal loss of plasma proteins into the gastrointestinal tract, it should now be possible to extend the techniques described in this thesis to a

study of the hypoproteinaemias associated with many intestinal parasitic diseases. Furthermore it is important that studies of this nature be combined with electron microscopic examination of the tissues of infected animals in order that a better understanding of the aetiology of abnormal protein loss be obtained.

Radioisotopes have also proved to be of considerable value in the study of anaemias which occur as a result of parasitic infections, and especially those in which loss of blood into the gastrointestinal tract is the primary influence, e.g. fascioliasis, hookworm disease, haemonchiasis. Most of the published data on parasitic anaemias has been obtained through the use of ^{51}Cr-labelled red cells, but although this label may be used to assess the importance of increased loss of red cells in causing anaemia (occurring as a result of either haemorrhage into the gut or excessive haemolysis), little information can be obtained concerning the erythropoietic response of the host to such losses. This is obviously very necessary because of the possible development of dyshaemopoiesis caused by a deficiency of some extrinsic factor as a result of the blood loss.

Much useful information concerning erythropoiesis can be obtained by using radioiron, but it should be borne in mind that the value of this technique (as in all others involving the use of isotopically labelled metabolites) is greatly enhanced when conventional analytical methods are also applied to the study. For instance, whereas the finding that intravenously injected ^{59}Fe disappeared from the plasma of fluke-infected rabbits

at a very much more rapid rate than from the controls is certainly very interesting, it is by itself virtually useless as far as yielding any new information concerning erythropoiesis in animals infected with this parasite, since it could be interpreted as indicating that such animals were iron deficient, or alternatively that the isotope was incorporated into a megaloblastic marrow.

Only when it was established that the serum iron levels of the infected animals were normal, and that the radioiron was in fact incorporated into new red cells which subsequently appeared in the peripheral blood very quickly was it possible to eliminate iron deficiency and impairment of erythropoiesis as secondary factors in causing the anaemia associated with this disease. Thus by combining ferrokinetic studies with those involving the use of ^{51}Cr-labelled red cells, it is possible not only to establish the primary influence in causing anaemia, but also to assess the importance of secondary factors in increasing its severity.

The nutritional consequences to the host of haemorrhage into the gastrointestinal tract is one aspect of parasitic infections which requires further investigation. The finding that iron lost into the alimentary tract as a result of haemorrhage is not significantly reabsorbed either in fluke-infected rabbits or sheep (Holmes, 1969), together with the increased amounts required for erythropoiesis, is bound to place an even heavier demand on the iron stores of the host.

It is also known that serum calcium and magnesium levels of fluke-infected sheep fall after the parasites enter the bile ducts (Sinclair 1962) and again it would appear that reabsorption of these elements is inadequate to meet body requirements. It is noteworthy in this respect that calcium and magnesium are necessary to maintain the structural and physiological integrity of cells and it may be that the altered mineral metabolism reported in parasitised animals facilitates leakage of protein between bile duct epithelial cells.

Clearly, loss of blood constituents into the gut whether reabsorbed or not will place a heavy demand on the host's absorptive and synthetic mechanisms. Survival of the host will thus depend not only upon the severity of the infection, but also upon the ability of the host to increase production of e.g. red cells and plasma proteins which will in turn be dependant upon the ready availability of amino acids, iron, etc. Where there is competition between host and parasite for essential nutrients (as occurs in Diphthlobothrium infection in man which causes anaemia due to the uptake of Vitamin B_{12} by the worm, thereby leading to a deprivation of the host,) the host's ability to survive infection will obviously be considerably reduced.

Since so little is known about the feeding habits, nutrition and physiology of many parasites, and the consequences of their presence to the host animal, it will be necessary to study more fully the basic physiology of the host-parasite relationship, and these investigations where possible, should be related to structural changes in the tissues of the host and the parasite.

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APPENDIX A

PLASMA PROTEIN METABOLISM IN NORMAL AND FLUKE-INFECTED RABBITS

^{131}I-LABELLED ALBUMIN TURNOVER STUDIES

DAILY FAECAL "CLEARANCES" OF PLASMA

Turnover of ^{131}I - Labelled Albumin in Normal and Fluke-infected Rabbits.

DAILY FAECAL RADIOACTIVITY EXPRESSED AS A PLASMA CLEARANCE.

RABBIT No. - 092 - Control.

Day of Expt.	Faecal Activity	Cumulative faecal activity	Cumulative faecal act. as % injected dose.	Plasma Clearance (ml).
1 and 2	256 + 262	517	0.33	-
3	744	1261	0.80	1.75
4	387	1648	1.05	0.97
5	342	1990	1.26	0.91
6	228	2218	1.41	0.64
7	232	2450	1.56	0.69
8	296	2746	1.74	0.95
9	38	2784	1.77	0.12
10	789	3573	2.27	2.84
11	168	3741	2.38	0.64
Mean				1.05
S.D.				0.80

Turnover of ^{131}I - labelled Albumin in Normal and Fluke-infected Rabbits.

DAILY FAECAL RADIOACTIVITY EXPRESSED AS A " PLASMA CLEARANCE ".

RABBIT NO. - 081 - Control.

Day of Expt.	Faecal Activity	Cumulative faecal activity	Cumulative activity as % injected act.	Plasma Clearance (ml).
1 and 2	216 + 285	501	0.32	-
3	295	796	0.51	0.73
4	224	1020	0.65	0.39
5	199	1219	0.77	0.56
6	176	1395	0.89	0.53
7	151	1546	0.98	0.49
8	113	1659	1.05	0.39
9	168	1827	1.16	0.63
10	72	1899	1.21	0.29
11	90	1989	1.26	0.39
Mean				0.51
S.D.				0.14

Turnover of ^{131}I - Labelled Albumin in Normal and Fluke-infected Rabbits

DAILY FAECAL RADIOACTIVITY EXPRESSED AS A " PLASMA CLEARANCE ".

RABBIT No. - 390 - Control.

Day of Expt.	Faecal Activity	Cumulative faecal activity	Cumulative faecal act. as % injected dose.	Plasma Clearance (ml).
1 and 2	190 + 300	490	0.31	-
3	742	1232	0.78	1.27
4	258	1490	0.95	0.49
5	204	1694	1.08	0.42
6	470	2164	1.37	1.06
7	433	2597	1.65	1.07
8	84	2681	1.70	0.22
9	108	2789	1.77	0.32
10	282	3071	1.95	0.91
11	107	3178	2.01	0.38
Mean				0.68
S.D.				0.39

Turnover of ^{131}I - Labelled Albumin in Normal and Fluke-infected Rabbits.

DAILY FAECAL RADIOACTIVITY EXPRESSED AS A PLASMA CLEARANCE.

RABBIT No. - 990 - Control.

Day of Expt.	Faecal Activity	Cumulative faecal activity	Cumulative faecal act. as % injected dose.	Plasma Clearance (ml.).
1 and 2	1088 + 1359	2447	1.95	-
3	511	2958	1.83	1.16
4	837	3795	2.41	2.11
5	955	4750	3.02	2.66
6	1079	5829	3.70	3.34
7	465	6294	4.00	1.58
8	308	6602	4.19	1.16
9	290	6892	4.38	1.20
10	180	7072	4.49	0.82
11	284	7356	4.67	1.11
Mean				1.68
S.D.				0.85

Turnover of ^{131}I - Labelled Albumin in Normal and Fluke-infected Rabbits.

DAILY FAECAL RADIOACTIVITY EXPRESSED AS A " PLASMA CLEARANCE ".

RABBIT No. - 083 - Infected.

Day of Expt.	Faecal Activity	Cumulative faecal activity	Cumulative activity as % injected act.	Plasma Clearance. (ml).
1 and 2	525 + 645	1170	-	-
3	508	1678	1.07	1.16
4	340	2018	1.28	0.90
5	658	2676	1.70	2.06
6	534	3210	2.04	2.04
7	827	4037	2.56	3.46
8	390	4427	2.81	1.89
9	243	4670	2.97	1.37
10	255	4925	3.13	1.67
11	120	5045	3.20	0.92
Mean				1.72
S.D.				0.79

Turnover of ^{131}I - Labelled Albumin in Normal and Fluke-infected Rabbits.

DAILY FAECAL RADIOACTIVITY EXPRESSED AS A " PLASMA CLEARANCE ".

RABBIT No.- 334 - Infected.

P

Day of Expt.	Faecal Activity	Cumulative faecal activity	Cumulative faecal act. as % injected dose.	Plasma Clearance (ml).
1 and 2	817 +90	907	0.57	-
3	235	1142	0.72	0.26
4	3360	4502	2.83	10.00
5	1269	5771	3.63	5.71
6	580	6351	3.99	3.25
7	384	6735	4.24	2.54
8	224	6959	4.38	1.81
9	278	7237	4.55	2.44
10	301	7538	4.74	3.75
11	220	7758	4.88	3.34
Mean				3.68
S.D.				2.79

Turnover of ^{131}I - Labelled Albumin in Normal and Fluke-infected Rabbits.

DAILY FAECAL RADIOACTIVITY EXPRESSED AS A " PLASMA CLEARANCE ".

RABBIT No. - 978 - Infected.

Day of Expt.	Faecal Activity	Cumulative faecal activity	Cumulative faecal act. as % injected dose	Plasma Clearance (ml).
1 and 2	460 + 564	1024	0.65	-
3	596	1620	1.03	1.63
4	259	1879	1.19	0.89
5	551	2430	1.54	2.45
6	360	2790	1.77	1.97
7	372	3162	2.00	2.41
8	272	3434	2.18	2.25
9	336	3770	2.39	3.32
10	351	4121	2.62	4.34
11	289	4410	2.80	4.68
Mean				2.70
S.D.				1.29

Turnover of ^{131}I - Labelled Albumin in Normal and Fluke-infected Rabbits

DAILY FAECAL RADIOACTIVITY EXPRESSED AS A " PLASMA CLEARANCE ".

RABBIT No. - 172 - Infected.

Day of Expt.	Faecal Activity	Cumulative faecal activity	Cumulative faecal act. as % injected dose.	Plasma Clearance (ml).
1 and 2	1037+=135	1172	0.74	-
3	837	2009	1.28	2.26
4	740	2749	1.75	2.58
5	461	3210	2.04	2.02
6	408	3618	2.30	2.00
7	460	4078	2.59	3.21
8	158	4236	2.69	1.40
9	451	4687	2.98	5.10
10	234	4921	3.12	3.33
11	167	5088	3.23	3.00
Mean				2.77
S.D.				1.08

^{131}I-LABELLED P.V.P. EXPERIMENTAL RESULTS

Use of ^{131}I - Labelled P.V.P. for Estimation of Movement of Plasma Macromolecules into the Gastro - Intestinal Tract of Normal and Fluke - Infected Rabbits.

PACKED CELL VOLUMES AND SERUM PROTEIN DATA.

	Rabbit No.	P.C.V.	Total Protein (gm%).	Albumin (gm%).	Globulin (gm%).	A/G	Globulin (gm%).		
							Alpha	Beta	Gamma
CONTROLS	985	34	5.66	2.76	2.90	0.95	0.68	1.19	1.03
	110	42	5.54	2.68	2.88	0.94	0.63	1.24	1.01
	119	39	6.71	2.98	3.73	0.80	0.51	1.87	1.35
	CLT	40	7.19	3.10	4.09	0.76	0.71	2.46	0.92
	203	35	5.49	2.57	2.92	0.88	0.42	1.32	1.18
	Mean	38	6.12	2.82	3.30	0.87	0.59	1.61	1.10
	S.D.	3.4	0.78	0.22	0.57	0.08	0.12	0.55	0.17
INFECTED	971	32	6.25	2.51	3.74	0.67	0.61	1.97	1.16
	084	20	4.69	1.72	2.97	0.58	0.55	1.42	1.00
	008	32	6.50	2.17	4.33	0.50	0.79	1.81	1.73
	996	29	5.26	2.32	2.94	0.79	0.62	1.36	0.96
	336	24	4.85	2.14	2.71	0.79	0.37	1.66	0.68
	Mean	27.4	5.51	2.17	3.34	0.67	0.59	1.64	1.11
	S.D.	5.3	0.82	0.29	0.68	0.13	0.15	0.26	0.39
	P.	< 0.01	> 0.10	< 0.01	> 0.10	< 0.02	> 0.10	> 0.10	> 0.10

Use of ^{131}I-labelled P.V.P. for Estimation of Movement of Plasma Macromolecules into the Intestinal Tract of Normal and Fluke-infected Rabbits

Daily ^{131}I-labelled P.V.P. Faecal Radioactivity Expressed as a "Plasma Clearance"

Day of Expt.	Control					Infected				
	985	110	199	CLT	203	971	084	088	996	336
1	2.20	1.34	4.78	2.56	5.68	22.18	6.20	11.44	23.97	22.81
2	2.23	5.92	5.88	2.18	5.73	30.84	9.01	18.34	26.25	27.47
3	1.84	2.02	4.18	3.27	5.05	24.97	20.90	29.47	32.80	30.92
4	2.21	4.02	3.70	2.84	4.59	27.33	14.72	22.34	40.24	39.50
5	2.96	2.96	5.27	3.50	4.14	27.09	34.10	41.97	28.52	36.67
Mean	2.29	3.25	4.76	2.87	5.04	26.48	16.99	24.71	30.36	31.47
S.D.	0.41	1.80	0.86	0.53	0.69	3.20	11.11	11.65	6.42	6.76

SIMULTANEOUS MEASUREMENTS OF THE TURNOVER OF ALBUMIN AND 7S GAMMAGLOBULIN

DISTRIBUTION AND CATABOLISM OF LABELLED PROTEINS

Albumin and 7S Gammaglobulin Turnover Studies in Normal and Fluke-Infected Rabbits
Packed Cell Volumes and Serum Protein Data

	Rabbit No.	P.C.V.	Total Protein (gms%)	Albumin (gms%)	Globulin (gms%)	A/G	Globulin (gms%)		
							Alpha	Beta	Gamma
CONTROL	429	44	6.24	3.56	2.68	1.33	0.52	1.36	0.80
	430	43	5.49	3.04	2.45	1.24	0.48	1.41	0.56
	433	37	5.20	2.37	2.83	0.83	0.60	1.81	0.42
	Mean	41.3	5.64	2.99	2.65	1.13	0.53	1.53	0.59
	S.D.	3.79	0.54	0.60	0.19	0.27	0.06	0.25	0.19
INFECTED	13	22	5.20	2.20	3.00	0.90	0.49	1.37	1.14
	23	27	5.50	2.29	3.21	0.71	0.57	1.49	1.15
	437	30	5.49	2.39	3.10	0.77	0.77	1.52	0.81
	313	32	5.61	3.06	2.55	1.20	0.60	1.26	0.69
	Mean	27.8	5.45	2.49	2.97	0.90	0.61	1.41	0.96
	S.D.	4.30	0.17	0.39	0.29	0.22	0.12	0.12	0.23
	P	<0.01	>0.10	>0.10	>0.10	>0.10	>0.10	>0.10	>0.10

Albumin and 7S Gammaglobulin Turnover Studies in Normal and Fluke-Infected Rabbits

Plasma Volumes

Rabbit No.	Plasma Volume	
	Ml.	Ml/Kg
429	77.29	32.89
430	74.06	31.25
433	75.14	38.53
Mean	75.49	34.22
S.D.	1.64	3.82
13	118.60	39.53
.23	100.60	35.17
437	69.10	39.04
313	64.30	33.85
Mean	88.15	36.90
S.D.	25.91	2.82
P	>0.10	>0.10

Turnover of ^{125}I-labelled Albumin/ ^{131}I-labelled 7S Gammaglobulin

in Normal and Fluke-infected Rabbits

Distribution of Gammaglobulin Between Vascular and Extravascular Pools

v Pool Sizes and Distribution Ratios(Campbell)

	Rabbit No.	CG (gm/kg)	EG (gm/kg)	TG (gm/kg)	EG/CG	EG/TG
CONTROL	429	0.26	0.40	0.66	1.50	0.60
	430	0.18	0.29	0.47	1.66	0.62
	433	0.16	0.20	0.36	1.25	0.56
	Mean	0.20	0.30	0.50	1.47	0.59
	S.D.	0.05	0.10	0.15	0.21	0.03
INFECTED	13	0.45	0.60	1.05	1.32	0.63
	23	0.41	0.41	0.82	1.01	0.50
	437	0.32	0.46	0.78	1.45	0.59
	313	0.23	0.35	0.58	1.50	0.60
	Mean	0.35	0.45	0.80	1.32	0.58
	S.D.	0.10	0.10	0.15	0.22	0.06
	P	<0.10	<0.10	<0.10	>0.10	>0.10

Turnover of ^{125}I-labelled Albumin/ ^{131}I-labelled 7S Gammaglobulin

in Normal and Fluke-infected Rabbits

F(CA) - Albumin Catabolic Rate (Campbell)

Day of	CONTROL			INFECTED			
Expt.	429	430	433	13	23	437	313
1	0.104	0.189	0.289	0.424	0.589	0.159	0.109
2	0.092	0.502	0.272	0.404	0.422	0.342	0.223
3	0.330	0.329	0.286	0.358	0.305	0.356	0.509
4	0.490	0.202	0.305	0.358	0.422	0.509	0.583
5	0.284	0.231	0.262	0.281	0.600	0.442	0.339
6	0.267	0.247	0.258	0.325	0.618	0.240	0.335
7	0.221	0.208	0.225	0.531	0.453	0.299	0.329
8	0.254	0.280	0.214	0.424	0.487	0.277	0.324
9	0.160	0.172	0.266	0.337	0.396	0.374	0.321
10	0.069	0.138	0.227	0.366	0.363	0.370	0.340
11	0.040	0.086	0.239	0.365	0.437	0.275	0.252
12	0.169	0.106	0.259	0.471	0.454	0.309	0.310
13	0.156	0.308	0.268	0.410	0.735	0.367	0.383
Mean	0.203	0.231	0.259	0.389	0.483	0.333	0.335
S.D.	0.123	0.109	0.024	0.065	0.119	0.087	0.117

Turnover of ^{125}I-labelled Albumin/ ^{131}I-labelled 7S Gammaglobulin in Normal and Fluke-infected Rabbits

F(CG) - Globulin Catabolic Rate (Campbell)

Day	Control			Infected			
	429	430	433	13	23	437	313
1	0.266	0.309	0.407	0.377	0.481	0.269	0.165
2	0.175	0.849	0.322	0.356	0.352	0.768	0.352
3	0.568	0.458	0.333	0.356	0.259	0.592	0.810
4	0.840	0.240	0.328	0.358	0.371	0.752	0.851
5	0.398	0.294	0.282	0.319	0.585	0.552	0.431
6	0.326	0.301	0.267	0.261	0.623	0.288	0.411
7	0.271	0.256	0.229	0.612	0.485	0.344	0.406
8	0.301	0.313	0.210	0.507	0.547	0.301	0.367
9	0.192	0.198	0.263	0.352	0.464	0.411	0.399
10	0.089	0.159	0.243	0.443	0.484	0.416	0.435
11	0.050	0.099	0.233	0.440	0.583	0.302	0.347
12	0.210	0.136	0.265	0.608	0.557	0.343	0.399
13	0.196	0.356	0.289	0.563	0.512	0.413	0.506
Mean	0.298	0.305	0.282	0.427	0.485	0.442	0.452
S.D.	0.210	0.190	0.055	0.113	0.104	0.171	0.185

Turnover of ^{125}I-labelled Albumin and ^{131}I-labelled 7S Gammaglobulin in Normal and Fluke-infected Rabbits

Daily ^{125}I (Albumin) Faecal Radioactivity Expressed as a "Plasma Clearance"

Day of Expt.	Control			Infected			
	429	430	433	13	23	437	313
3	1.11	0.69	0.77	2.46	2.35	1.86	4.65*
4	0.44	0.45	0.68	4.53	3.60	2.87	4.83*
5	0.75	0.36	0.52	2.97	3.46	2.72	1.66
6	1.00	0.43	0.36	2.78	4.39	2.15	1.73
7	0.71	0.36	0.35	1.56	3.14	1.88	1.48
8	0.59	0.54	0.41	2.52	4.90	1.94	1.54
9	0.79	0.89	0.40	2.47	3.27	2.15	0.94
10	0.49	1.06	0.39	3.62	2.67	1.71	1.10
11	0.50	1.03	0.49	2.86	4.05	2.46	1.15
12	0.43	0.10	0.38	3.07	1.00	1.88	0.94
13	0.83	0.47	0.40	3.93	4.83	2.48	0.94
Mean	0.69	0.58	0.47	2.98	3.43	2.19	1.32
S.D.	0.23	0.30	0.14	0.81	1.15	0.39	0.11

* - Contaminated Faeces.

Turnover of ^{125}I-labelled Albumin / ^{131}I-labelled 7S Gammaglobulin in Normal and Fluke-infected Rabbits

Daily ^{131}I (Globulin) Faecal Radioactivity Expressed as a " Plasma Clearance"

Day of Expt.	Control			Infected			
	429	430	433	13	23	437	313
3	1.90	1.03	1.34	4.40	2.56	5.65*	3.86*
4	0.65	0.75	1.15	3.69	2.43	0.20*	7.50*
5	0.47	0.60	0.89	3.83	3.63	3.69	2.50
6	0.78	0.65	0.60	2.58	3.52	2.68	2.37
7	1.00	0.58	0.62	3.59	4.48	2.37	2.23
8	0.95	0.79	0.81	4.06	3.17	2.26	2.14
9	1.24	1.10	0.87	2.78	4.91	2.41	1.74
10	0.80	1.19	0.76	5.63	3.46	1.93	1.50
11	0.78	1.19	0.93	5.75	2.85	2.68	1.67
12	0.56	0.12	0.81	2.95	4.26	2.10	1.85
13	1.08	0.55	0.57	2.95	3.15	2.79	3.57*
Mean	0.92	0.78	0.85	3.84	3.49	2.55	2.00
S.D.	0.39	0.33	0.23	1.08	0.79	0.51	0.36

* Contaminated Faeces

APPENDIX B

THE ANAEMIA OF FASCIOLIASIS IN THE RABBIT

STUDIES WITH ^{51}Cr and ^{59}Fe-LABELLED ERYTHROCYTES

^{51}Cr-LABELLED RED CELL TURNOVER STUDIES

DAILY FAECAL "CLEARANCES" OF WHOLE BLOOD AND RED CELLS

Turnover of ^{51}Cr - Labelled Erythrocytes in Normal and Fluke-infected Rabbits.

DAILY FAECAL RADIOACTIVITY EXPRESSED AS A CLEARANCE OF BLOOD AND RED CELLS.

RABBIT No. - 978 - INFECTED.

Day of Expt.	Total Faecal Activity.	Cumulative Faecal Activity	Cumulative faecal activity as % injected dose	Blood Clearance (ml)	Red Cell Clearance (ml)
1	499	-	-	-	-
2	1119	1618	6.3	6.8	1.7
3	1176	2794	10.5	9.6	2.3
4	1706	4500	16.9	18.6	4.3
5	2593	7093	26.7	37.7	8.4
6	2361	9454	35.5	45.6	9.9
7	1150	10604	39.9	29.9	6.2
8	967	11571	43.5	33.7	6.7
9	564	12135	45.6	26.0	5.0
Mean				25.99	5.56
S.D.				13.56	2.83

Turnover of ^{51}Cr - Labelled Erythrocytes in Normal and Fluke-infected Rabbits.

DAILY FAECAL RADIOACTIVITY EXPRESSED AS A "CLEARANCE" OF BLOOD AND RED CELLS.

RABBIT No. - OC8 - INFECTED.

Day of Expt.	Total Faecal Activity	Cumulative Faecal Activity	Cumulative faecal Activity as % injected dose.	Blood Clearance (ml).	Red Cell Clearance (ml).
1	148	-	-	-	-
2	238	386	1.8	1.3	0.5
3	400	786	3.6	2.3	0.8
4	485	1271	5.9	3.1	1.0
5	526	1797	8.3	3.7	1.2
6	614	2411	11.2	4.8	1.6
7	430	2841	13.2	3.7	1.3
8	313	3154	14.6	3.0	1.0
9	242	3396	15.7	2.6	0.9
Mean				3.06	1.04
S.D.				1.06	0.33

Turnover of ^{51}Cr - Labelled Erythrocytes in Normal and Fluke-infected Rabbits.

DAILY FAECAL RADIOACTIVITY EXPRESSED AS A "CLEARANCE" OF BLOOD AND RED CELLS.

RABBIT No. - 083 - INFECTED.

Day of Expt.	Total Faecal Activity.	Cumulative Faecal Activity.	Cumulative faecal Activity as % injected dose.	Blood Clearance (ml).	Red Cell Clearance (ml).
1	638	-	-	-	-
2	1368	2006	12.4	12.7	2.7
3	1407	3413	21.1	16.8	3.7
4	973	4386	27.1	14.9	3.3
5	804	5190	32.0	15.6	3.6
6	565	5755	35.5	14.0	3.3
7	406	6161	38.0	13.0	3.1
8	467	6628	40.9	19.3	4.8
9	262	6890	42.5	14.9	3.2
Mean				15.15	3.46
S.D.				2.15	0.62

Turnover of ^{51}Cr - Labelled Erythrocytes in Normal and Fluke-infected Rabbits.

DAILY FAECAL RADIOACTIVITY EXPRESSED AS A "CLEARANCE" OF BLOOD AND RED CELLS.

RABBIT No. - 179 - INFECTED.

Day of Expt.	Total Faecal Activity.	Cumulative Faecal Activity.	Cumulative faecal Activity as % injected dose.	Blood Clearance (ml).	Red Cell Clearance (ml.)
1	157	-	-	-	-
2	522	679	5.17	9.7 x	1.6 x
3	1508	2187	18.4	40.2	6.6
4	1171	3358	28.2	47.0	7.6
5	807	4165	35.0	48.6	7.8
6	640	4805	40.4	57.7	9.1
7	347	5152	43.4	47.5	7.3
8	223	5375	45.2	45.5	6.9
9	149	5524	46.4	45.2	6.9
Mean				47.39	7.46
S.D.				5.29	0.84.

x - Excluded in Calculation of Mean Clearances.

Turnover of ^{51}Cr - Labelled Erythrocytes in Normal and Fluke-infected Rabbits.

DAILY FAECAL RADIOACTIVITY EXPRESSED AS A "CLEARANCE" OF BLOOD AND RED CELLS.

RABBIT NO. - 590- CONTROL.

Day of Expt.	Total Faecal Activity.	Cumulative Faecal Activity.	Cumulative Activity as % injected dose.	Blood Clearance (ml).	Red Cell Clearance (ml).
1	19	-	-	-	-
2	85	104	0.36	0.31	0.11
3	35	139	0.48	0.15	0.05
4	52	191	0.66	0.24	0.08
5	61	252	0.87	0.31	0.10
6	30	282	0.97	0.16	0.05
7	30	312	1.10	0.18	0.09
8	18	330	1.13	0.12	0.04
9.	14	3.44	1.18	0.10	0.03
Mean				0.20	0.065
S.D.				0.08	0.028

Turnover of ^{51}Cr - Labelled Erythrocytes in Normal and Fluke-infected Rabbits.

DAILY FAECAL RADIOACTIVITY EXPRESSED AS A "CLEARANCE" OF BLOOD AND RED CELLS.

RABBIT NO. - 082 - CONTROL.

Day of Expt.	Total Faecal Activity.	Cumulative Faecal Activity	Cumulative Activity as % injected dose.	Blood Clearance (ml).	Red Cell Clearance (ml).
1	8	-	-	-	-
2	10	18	0.11	0.08	0.03
3	12	30	0.18	0.11	0.04
4	9	39	0.24	0.09	0.03
5	7	46	0.28	0.07	0.02
6	8	54	0.33	0.09	0.03
7	9	63	0.39	0.11	0.04
8	18	81	0.50	0.24	0.08
9	66	147	0.92	0.96	0.30
Mean				0.22	0.069
S.D.				0.30	0.093

Turnover of ^{51}Cr - Labelled Erythrocytes in Normal and Fluke-infected Rabbits.

DAILY FAECAL RADIOACTIVITY EXPRESSED AS A "CLEARANCE" OF BLOOD AND RED CELLS.

RABBIT NO. - C81 - CONTROL.

Day of Expt.	Total Faecal Activity.	Cumulative Faecal Activity	Cumulative Activity as % injected dose.	Blood Clearance (ml).	Red Cell Clearance (ml).
1	6	-	-	-	-
2	20	26	0.14	0.16	0.06
3	16	42	0.23	0.14	0.05
4	16	58	0.31	0.15	0.05
5	24	82	0.44	0.26	0.09
6	12	94	0.51	0.14	0.05
7	14	108	0.58	0.18	0.05
8	11	119	0.64	0.16	0.05
9	7	126	0.68	0.11	0.04
Mean				0.16	0.056
S.D.				0.04	0.014

Turnover of ^{51}Cr - Labelled Erythrocytes in Normal and Flaker-infected Rabbits.

DAILY FAECAL RADIOACTIVITY EXPRESSED AS A "CLEARANCE" OF BLOOD AND RED CELLS.

RABBIT NO. - 220 - CONTROL.

Day of Expt.	Total Faecal Activity.	Cumulative Faecal Activity	Cumulative Activity as % injected dose	Blood Clearance (ml).	Red Cell Clearance (ml).
1	1	-	-	-	-
2	19	20	0.08	0.09	0.03
3	46	66	0.24	0.23	0.09
4	23	89	0.33	0.13	0.05
5	46	135	0.51	0.28	0.11
6	29	164	0.62	0.20	0.07
7	50	214	0.80	0.38	0.14
8	29	243	0.91	0.24	0.09
9	33	276	1.04	0.31	0.11
Mean				0.23	0.086
S.D.				0.09	0.039

^{59}Fe-LABELLED RED CELL TURNOVER STUDIES

DAILY FAECAL "CLEARANCES" OF WHOLE BLOOD AND RED CELLS

^{59}Fe Labelled R.B.C. Turnover Studies in Normal and Fluke-infected Rabbits

$T_{\frac{1}{2}}$ (hours) of ^{59}Fe Labelled Red Cells

	Rabbit No.	Half Life (hours)	Flukes Recovered
INFECTED	306	75	27
	416	390	7
	315	99	22
	363	90	28
	177	90	25
	280	200	16
	Mean	157.3	-
	S.D.	122.6	-
CONTROL	377	1834	-
	361	1309	-
	Mean	1571.5	-
	S.D.	371.2	-
	P	<0.001	-

Rabbit No. 306 - Infected

Day	Total ^{59}Fe Faecal Act.	Cumulative Faecal Activity	Cumulative Faecal as % Inj.	Blood Clearance (ml/24hrs)	R.B.C. Clearance (ml/24hrs)	Haemoglobin Iron Excreted (mg/24hrs)
1	5,383		3.31			
2	8,257	13,640	8.39	5.50	1.13	0.98
3	21,467	35,107	21.60	17.52	3.58	3.12
4	13,532	48,639	29.93	13.67	2.88	2.12
5	11,014	59,653	36.71	14.12	2.89	2.47
6	18,529	78,182	48.11	29.89	6.18	3.60
7	7,413	85,595	52.67	15.13	3.09	2.68
8	4,882	90,477	55.67	12.52	2.57	2.45
9	6,925	97,402	59.93	22.34	4.62	4.38
10	3,493	100,895	62.08	13.97	2.91	3.17
11	3,137	104,032	64.01	16.09	3.27	3.65
12	1,838	105,970	65.14	11.86	2.42	2.05
13	1,557	107,427	66.10	12.46	2.59	2.18
14	1,102	108,529	66.78	11.02	2.29	2.42
15	1,764	110,293	67.87	21.51	4.64	4.73
16	1,208	111,501	68.61	18.03	4.02	3.97
17	1,034	112,535	69.25	19.88	4.31	3.70
18	788	113,323	69.73	17.91	4.15	3.33
19	569	113,892	70.08	16.26	3.79	3.02
20	484	114,376	70.38	16.13	4.03	3.19
21	574	114,950	70.73	23.92	6.04	4.74

Rabbit No. 416 - Infected

Day	Total ^{59}Fe Faecal Act.	Cumulative Faecal Activity	Cumulative Faecal Act. as % Inj.	Blood Clearance (ml/24 hrs)	R.B.C. Clearance (ml/24hrs)	Haemoglobin Iron Excreted (mg/24hrs)
1	1,354		0.83			
2	2,117	3,471	2.13	1.84	0.62	0.80
3	4,138	7,609	4.67	3.76	1.29	1.64
4	4,915	12,524	7.69	4.68	1.64	1.63
5	4,613	17,137	10.53	4.61	1.65	1.69
6	4,623	21,760	13.37	4.92	1.81	1.59
7	4,515	26,275	16.14	5.02	1.77	1.78
8	3,309	29,584	18.17	3.85	1.38	1.58
9	3,238	32,822	20.16	3.95	1.41	1.62
10	2,890	35,712	21.94	3.71	1.34	1.58
11	2,674	38,386	23.58	3.61	1.32	1.53
12	3,510	41,896	25.73	5.01	1.85	1.86
13	3,752	45,648	28.04	5.68	2.08	2.06
14	3,236	48,884	30.03	5.14	1.90	2.21
15	2,449	51,333	31.53	4.08	1.53	1.75
16	3,047	54,380	33.40	5.35	2.00	2.30
17	2,399	56,779	34.87	4.44	1.67	1.70
18	2,343	59,122	36.31	4.51	1.72	1.73
19	3,008	62,130	38.16	6.14	2.35	2.36
20	2,136	64,266	39.47	4.54	1.78	1.78
21	1,968	66,234	40.68	4.37	1.71	1.73

Rabbit No. 363 - Infected

Day	Total ^{59}Fe Faecal Activity	Cumulative Faecal Activity	Cumulative Faecal as % Ini.	Blood Clearance (ml/24hrs)	R.B.C. Clearance (ml/24hrs)	Haemoglobin Iron Excreted (mg/24hrs)
1	1,916		1.15			
2	11,852	13,768	8.25	9.12	2.28	2.89
3	10,909	24,677	14.79	9.74	2.42	3.09
4	13,832	38,509	23.08	14.71	3.74	3.70
5	13,105	51,614	30.93	16.80	4.23	4.25
6	10,614	62,228	37.29	16.08	4.08	3.94
7	10,999	73,227	43.88	19.99	5.12	5.05
8	6,807	80,034	47.96	14.79	3.78	4.20
9	6,167	86,201	51.65	15.81	4.17	4.49
10	4,814	91,015	65.65	14.81	3.91	4.12
11	2,102	93,117	55.80	7.79	2.10	2.17
12	3,548	96,665	57.92	15.43	4.22	3.63
13	3,557	100,222	60.06	18.72	5.09	4.46
14	2,399	102,621	61.49	14.99	4.14	3.12
15	1,871	104,492	62.61	13.86	3.89	2.88
16	1,711	106,203	63.64	15.55	4.28	3.23
17	1,341	107,544	64.44	14.90	4.06	3.50
18	1,271	108,815	65.20	16.95	4.71	3.98
19	536	109,351	65.53	8.65	2.33	2.03
20	419	109,770	65.78	7.48	2.21	1.76
21	-					

Rabbit No. 315 - Infected

Day	Total ^{59}Fe Faecal Activity	Cumulative Faecal Activity	Cumulative Faecal as % Inj.	Blood Clearance (ml/24hrs)	R.B.C. Clearance (ml/24hrs)	Haemoglobin Iron Excreted (mg/24hrs)
1	2,121		1.34			
2	8,655	10,776	6.81	5.77	1.25	1.60
3	14,099	24,875	15.72	10.85	2.39	3.00
4	10,524	35,399	22.37	9.48	2.10	1.77
5	11,573	46,972	29.68	12.06	2.76	2.51
6	8,764	55,736	35.22	10.82	2.50	3.21
7	8,367	64,103	40.51	12.13	2.79	3.99
8	6,728	70,831	44.76	11.40	2.69	2.82
9	5,186	76,017	48.04	10.37	2.47	2.56
10	2,685	78,702	49,73	6.24	1.49	1.38
11	3,545	82,247	51.97	9.58	2.36	2.12
12	2,244	84,491	53.39	7.24	1.75	1.53
13	3,618	88,109	55.68	13.65	3.38	3.07
14	3,335	91,444	57.78	14.50	3.71	3 38
15	1,698	93,142	58.86	8.71	2.23	2.03
16	1,912	95,054	60.07	11.59	2.99	2.70
17	1,855	96,909	61.24	13.25	3.50	3.11
18	1,108	98,017	61.94	9.23	2.46	2.17
19	625	98,642	62.33	5.95	1.64	1.40
20	1,133	99,775	63,05	12.59	3.54	2.96
21	1,033	100,808	63.70	12.91	3.83	3.03

Rabbit No. 177 - Infected

Day	Total ^{59}Fe Faecal Activity	Cumulative Faecal Activity	Cumulative Faecal as % Inj.	Blood Clearance (ml/24hrs)	R.B.C. Clearance (ml/24hrs)	Haemoglobin Iron Excreted (mg/24hrs)
1	3,515		2.15			
2	9,397	12,912	7.88	8.17	2.04	1.64
3	12,746	26,658	15.67	12.75	3.35	2.56
4	9,386	35,044	21.40	11.17	2.93	2.59
5	9,392	44,436	27.13	13.42	3.48	3.05
6	9,212	53,648	32.76	15.61	4.19	5.45
7	7,394	61,042	37.27	14.79	3.99	3.74
8	5,389	66,431	40.56	12.83	3.52	3.89
9	4,207	70,638	43.13	12.02	3.29	3.64
10	3,664	74,302	45.37	12.42	3.42	3.29
11	2,722	77,024	47.03	10.89	3.09	3.89
12	3,295	80,319	49.04	15.69	4.45	3.89
13	1,929	82,248	50.22	11.02	3.11	3.17
14	2,083	84,331	51.49	13.89	3.93	3.24
15	1,593	85,924	52.46	12.74	3.70	2.97
16	1,436	87,360	53.34	13.68	4.10	3.16
17	945	88,305	53.92	10.50	3.26	2.60
18	1,115	89,420	54.60	15.93	4.65	3.95
19	658	90,078	55.00	10.12	3.29	2.51
20	277	90,355	55.17	4.61	1.63	1.25
21	427	90,782	55.43	8.54	3.05	1.47

Rabbit No. 280 - Infected

Day	Total ^{59}Fe Faecal Activity	Cumulative Faecal Activity	Cumulative Faecal as % Inj.	Blood Clearance (ml/24hrs)	R.B.C. Clearance (ml/24hrs)	Haemoglobin Iron Excreted (mg/24hrs)
1	662					
2	5,134	5,796	3.63	4.19	1.35	1.55
3	9,543	15,339	9.60	8.20	2.73	3.06
4	7,712	23,051	14.43	7.34	2.41	2.09
5	7,277	30,328	18.99	7.58	2.51	2.30
6	5,873	36,201	22.66	6.60	2.19	1.96
7	8,844	45,045	28.20	11.06	3.65	2.55
8	6,849	51,894	32.49	9.26	3.11	3.05
9	4,986	56,880	35.61	7.33	2.49	2.41
10	3,987	60,867	38.10	6.43	2.18	1.78
11	3,259	64,126	40.14	5.72	1.93	1.59
12	4,179	68,305	42.76	7.88	2.73	2.54
13	3,031	71,336	44.66	6.31	2.17	2.13
14	4,043	75,379	47.19	9.19	3.16	2.94
15	3,784	79,163	49.56	9.23	3.23	2.95
16	2,987	82,150	51.43	7.97	2.84	2.55
17	2,814	84,964	53.19	8.16	2.93	2.63
18	2,282	87,246	54.62	7.13	2.59	2.30
19	2,865	90,111	56.41	9.88	3.58	3.18
20	1,988	92,099	57,65	7.50	2.69	2.42
21	2,671	94,770	59.33	10.90	4.05	3.51

Rabbit No. 377 - Control

Day	Total ^{59}Fe Faecal Activity	Cumulative Faecal Activity	Cumulative Faecal as % Inj.	Blood Clearance (ml/24hrs)	R.B.C. Clearance (ml/24hrs)	Haemoglobin Iron Excreted (mg/24hrs)
1	663		0.40			
2	426	1,089	0.65	0.59	0.22	0.31
3	978	2,067	1.24	1.36	0.52	0.72
4	772	2,839	1.71	1.07	0.41	0.61
5	1,593	4,432	2.66	2.21	0.87	0.95
6	1,604	6,036	3.63	2.23	0.89	1.09
7	1,324	7,360	4.42	1.84	0.74	0.95
8	762	8,122	4.88	1.06	0.42	0.50
9	1,152	9.274	5.57	1.60	0.64	0.76
10	654	9,928	5.97	0.9[illegible]	0.37	0.44
11	452	10,380	6.24	0.63	0.26	0.30
12	644	11,024	6.63	0.89	0.38	0.40
13	854	11,878	7.14	1.19	0.50	0.54
14	533	12,411	7.46	0.74	0.31	0.34
15	370	12,781	7.68	0.51	0.22	0.23
16	327	13,108	7.88	0.45	0.19	0.21
17	282	13,390	8.05	0.39	0.17	0.18
18	185	13,575	8.16	0.26	0.11	0.12
19	173	13,748	8.26	0.24	0.11	0.11
20	286	14,034	8.44	0.39	0.18	0.18
21	229	14,263	8.57	0.32	0.14	0.15

Rabbit No. 361 - Control

Day	Total ^{59}Fe Faecal Activity	Cumulative Faecal Activity	Cumulative Faecal as % Inj.	Blood Clearance (ml/24hrs)	R.B.C. Clearance (ml/24hrs)	Haemoglobin Iron Excreted (mg/24hrs)
1	234		0.16			
2	782	1,016	0.67	0.62	0.23	0.30
3	811	1,827	1.25	0.65	0.24	0.31
4	712	2,539	1.74	0.58	0.21	0.25
5	555	3,094	2.12	0.46	0.17	0.20
6	684	3,778	2.59	0.57	0.21	0.28
7	461	4,239	2.91	0.39	0.14	0.20
8	722	4,961	3.40	0.62	0.23	0.26
9	400	5,361	3.67	0.35	0.13	0.15
10	262	5,623	3.85	0.23	0.09	0.10
11	204	5,827	3.99	0.18	0.07	0.08
12	402	6,229	4.27	0.37	0.14	0.17
13	254	6,483	4.44	0.23	0.09	0.09
14	165	6,648	4.56	0.15	0.06	0.06
15	167	6,815	4.67	0.16	0.06	0.06
16	136	6,951	4.76	0.13	0.05	0.05
17	205	7,156	4.91	0.20	0.07	0.08
18	133	7,289	5.00	0.13	0.05	0.05
19	134	7,423	5.09	0.14	0.05	0.06
20	114	7,537	5.17	0.12	0.04	0.05
21	193	7,730	5.30	0.20	0.07	0.08

^{59}Fe-Labelled R.B.C. Turnover Studies in Normal and Fluke-infected Rabbits

Faecal Excretion of ^{59}Fe

	Rabbit No.	Cumulative Faecal Activity as % Inj. (21 days)
INFECTED	306	70.73
	416	40.68
	315	63.70
	363	65.78
	177	55.43
	280	59.33
	Mean	59.28
	S.D.	10.52
CONTROL	377	8.57
	361	5.30
	Mean	6.94
	S.D.	2.31
	P	< 0.001

INTESTINAL LOSS AND REABSORPTION OF IRON IN NORMAL AND FLUKE-INFECTED RABBITS

SIMULTANEOUS USE OF ^{59}Fe and ^{51}Cr-LABELLED ERYTHROCYTES

DAILY FAECAL "CLEARANCES" OF WHOLE BLOOD AND RED CELLS

^{59}Fe / ^{51}Cr-labelled Red Cell Turnover Studies in 4n Normal and Fluke-infected Rabbits
Erythrocyte Studies

	Rabbit No.	Packed Red Cells (%)	Hb (g/100 ml blood)	R.B.C. Count ($\times 10^6$ cu.mm)	M.C.V. (cu.μ)	M.C.H.C. (%)
INFECTED	340	26	6.76	2.72	95.6	26
	334	28	10.34	4.18	67.0	36.9
	B1	25	7.11	3.40	73.5	28.4
	339	31	9.35	3.68	84.2	30.2
	335	25	6.88	2.85	87.7	27.5
	316	29	8.47	3.24	89.5	29.2
	Mean	27.3	8.15	3.35	82.9	29.7
	S.D.	2.4	1.48	0.54	10.7	3.8
CONTROL	342	38	13.52	5.64	67.4	35.6
	201	35	15.24	4.93	71.0	43.5
	179	38	12.73	5.46	69.6	33.5
	344	39	13.52	5.38	72.5	34.7
	Mean	37.5	13.75	5.35	70.1	36.8
	S.D.	1.7	1.06	0.30	2.2	4.5
	P	< 0.001	< 0.001	< 0.001	< 0.05	$< 0;05$

Turnover of ^{59}Fe and ^{51}Cr Labelled Red Cells in Normal and Fluke-Infected Rabbits

$T_{\frac{1}{2}}$ (hours) of ^{59}Fe and ^{51}Cr Labelled R.B.C's

	Rabbit No.	^{59}Fe	^{51}Cr
INFECTED	316	69	55
	B1	105	71
	340	125	95
	334	112	87
	335	94	63
	339	192	136
	Mean	116.2	84.5
	S.D.	41.7	29.3
CONTROL	344	805	237
	179	776	228
	201	803	216
	342	1287	293
	Mean	917.8	243.5
	S.D.	246.5	34.1
	P	<0.001	<0.001

Rabbit No. 339 - Infected

Day	Total ^{59}Fe Faecal Activity	^{59}Fe Blood Clearance (ml/24hrs)	^{59}Fe R.B.C. Clearance (ml/24hrs)	Total ^{51}Cr Faecal Clearance	^{51}Cr Blood Clearance (ml/24hrs)	^{51}Cr R.B.C. Clearance (ml/24hrs)	Haemoglobin Fe/24hrs Lost into gut (mg)	Haemoglobin Fe/24hrs Excreted (mg)
1	3,536							
2	10,638	3.9	1.10					1.32
3	8,045	3.2	0.90					1.04
4	13,277	5.7	1.63					1.86
5	12,185	5.7	1.63	2,591				1.96
6	9,715	4.9	1.42	11,174	3.80	1.11	1.15	1.49
7	6,728	3.6	1.07	12,377	4.74	1.40	1.29	0.98
8	5,997	3.5	1.05	13,198	5.61	1.69	1.84	1.15
9	10,773	6.7	2.04	12,673	6.06	1.84	1.73	1.91
10	6,183	4.2	1.28	9,900	5.32	1.62	1.92	1.52
11	6,862	5.0	1.55	7,858	4.72	1.46	1.71	1.81
12	6,142	4.9	1.52	7,601	5.12	1.59	1.77	1.70
13	4,056	3.5	1.09	5,688	4.28	1.35	1.31	1.07
14	2,295	2.1	0.67	3,651	3.11	0.98	1.08	0.73
15	3,812	3.8	1.21	2,916	2.79	0.89	1.01	1.38
16	3,330	3.7	1.15	2,453	2.59	0.84	0.98	1.40
17	6,836	8.0	2.60	6,127	7.28	2.39	2.60	2.86
18	6,046	7.8	2.47	4,070	5.42	1.79	1.59	2.29
19	5,837	8.3	2.61	3,769	5.63	1.87	1.57	2.31

Rabbit No. 335 - Infected

Day	Total ^{59}Fe Faecal Activity	^{59}Fe Blood Clearance (ml/24hrs)	^{59}Fe R.B.C. Clearance (ml/24hrs)	Total ^{51}Cr Faecal Activity	^{51}Cr Blood Clearance (ml/24hrs)	^{51}Cr R.B.C. Clearance (ml/24hrs)	Haemoglobin/24hrs. Lost into gut (mg)	Haemoglobin/24hrs. Excreted (mg)
1	11,086							
2	12,758	5.9	1.26					0.56
3	22,572	12.2	2.54					0.53
4	25,164	16.0	3.36					0.95
5	17,729	13.6	2.83	9.235				0.98
6	12,775	11.6	2.41	18,613	9.91	1.96	0.70	0.91
7	9,904	10.8	2.24	21.092	14.33	2.92	0.74	0.56
8	5,384	6.9	1.46	13,614	11.92	2.44	0.88	0.55
9	7,171	11.2	2.31	10,096	11.37	2.34	1.20	1.33
10	4,998	9.3	1.92	8,052	11.75	2.44	1.03	0.81
11	5,981	13.3	2.74	6,749	12.66	2.66	1.00	1.06
12	5,796	15.3	3.18	6,737	16.08	3.43	0.96	0.92
13	4,481	14.0	2.95	4,640	14.41	3.07	1.07	0.87
14	2,765	10.43	2.16	3,734	15.00	3.20	0.73	0.50
15	3,467	15.80	3.24	3,985	20.44	4.49	0.57	0.78
16	2,213	11.9	2.46	1,702	11.20	2.48	0.47	0.68
17	2,558	16.5	3.41	1 ,076	9.04	2.02	1.30	1.43
18	2,082	16.0	3.30	1,079	11.86	2.66	0.97	1.40
19	1,561	14.2	2.95	873	12.30	2.75	1.16	1.70

Rabbit No. 340 - Infected

Day	Total ^{59}Fe Faecal Activity	^{59}Fe Blood Clearance (ml/24hrs)	^{59}R.B.C. Clearance (ml/24hrs)	Total ^{51}Cr Faecal Activity	^{51}Cr Blood Clearance (ml/24hrs)	^{51}Cr R.B.C. Clearance (ml/24hrs)	Haemoglobin Fe/24hrs. Lost into gut (mg)	Haemoglobin Fe/24hrs. Excreted (mg)
1	4,998							
2	17,253	6.5	1.61					1.49
3	5,842	2.5	0.62					0.56
4	18,639	9.3	2.25					2.09
5	17,617	10.1	2.48	7,243				2.64
6	16,375	10.7	2.57	16,170	7.68	1.87	1.84	2.57
7	10,064	7.6	1.80	15,936	9.09	2.18	2.11	1.77
8	10,605	9.2	2.16	17,283	11.89	2.85	2.94	2.28
9	13,209	13.2	3.10	14,372	11.95	2.82	2.44	2.70
10	10,361	12.0	2.76	13,356	13.32	3.16	3.49	3.14
11	9,159	12.1	2.78	10,816	13.08	3.05	3.91	3.62
12	8,635	13.1	3.02	9,127	13.25	3.01	3.47	3.43
13	6,060	10.6	2.42	6 ,404	11.10	2.55	3.69	3.52
14	4,354	8.7	1.98	6,814	14.32	3.26	4.66	2.83
15	5,980	13.9	3.08	3,689	9.36	2.08	2.78	4.13
16	2,806	7.6	1.65	1,709	5.24	1.17	1.42	2.06
17	9,855	30.8	6.57	6,335	23.37	5.14	5.22	6.88
18	-	-	-	-	-	-	-	-
19	5,510	22.9	4.83	3,240	17.42	3.78	4.16	5.47

Rabbit No. 316 - Infected

Day	Total ^{59}Fe Faecal Activity	^{59}Fe Blood Clearance (ml/24hrs)	^{59}Fe R.B.C. Clearance (ml/24hrs)	Total ^{51}Cr Faecal Activity	^{51}Cr Blood Clearance (ml/24hrs)	^{51}Cr R.B.C. Clearance (ml/24hrs)	Haemoglobin Fe/24hrs Lost into gut (mg)	Haemoglobin Fe/24hrs Excreted (mg)
1	7,830							
2	25,399	9.44	1.55					2.09
3	21,849	9.9	1.69					1.57
4	20,214	11.6	2.02					1.84
5	24,292	17.4	3.11	5,822				2.87
6	13,272	11.8	2.16	13,866	11.33	2.27	1.87	1.95
7	11,474	12.8	2.39	10,800	11.97	2.40	2.01	2.15
8	3,640	5.1	0.97	7,858	11.75	2.38	2.52	1.09
9	10,382	18.2	3.52	5,702	11.43	2.35	2.36	3.76
10	6,928	15.1	3.01	6,808	18.35	3.82	3.66	3.00
11	3,374	9.1	1.87	3,598	13.08	2.74	3.42	2.38
12	2,628	8.9	1.84	3,427	16.4	3.58	3.48	1.89
13	4,887	20.8	4.40	3,169	20.44	4.56	4.70	4.78
14	2,378	12.5	2.73	2,772	23.90	5.27	4.88	2.55
15	2,332	15.5	3.43	952	11.20	2.49	2.22	3.07
16	2,541	21.2	4.75	1,069	16.97	3.82	3.43	4.29
17	2,074	21.6	4.96	911	19.38	4.38	3.59	4.00
18	1,380	16.6	4.25	511	14.60	3.34	2.66	3.02
19	1,134	14.4	4.45	524	20.15	4.60	3.56	2.55

Rabbit No. Blackie - Infected

Day	Total ^{59}Fe Faecal Activity	^{59}Fe Blood Clearance (ml/24hrs)	^{59}Fe R.B.C. Clearance (ml/24hrs)	Total ^{51}Cr Faecal Activity	^{51}Cr Blood Clearance (ml/24hrs)	^{51}Cr R.B.C. Clearance (ml/24hrs)	Haemoglobin Fe/24hrs	
							Lost into gut (mg)	Excreted (mg)
1	595							
2	6,114	3.7	0.63					0.71
3	35,541	24.8	4.23					3.86
4	10,974	8.8	1.53					1.37
5	18,020	16.8	2.95	2,010				2.55
6	13,060	13.8	2.51	5,810	5.72	1.13	0.60	1.46
7	14,293	17.3	3.23	14,006	17.19	3.43	2.62	2.64
8	8,804	12.2	2.32	11,359	17.53	3.50	3.26	2.27
9	18,483	29.3	5.76	13,414	25.80	5.21	4.47	5.08
10	9,223	16.8	3.40	8,333	20.03	4.12	3.24	2.72
11	1,373	2.9	0.58	1,039	3.12	0.65	0.60	0.56
12	6,002	14.9	3.0	3,954	14.70	3.18	2.29	2.32
13	7,360	20.4	4.33	4,704	22.08	4.73	3.90	3.60
14	1,232	4.0	0.85	1,224	7.16	1.57	1.43	0.80
15	8,126	30.0	6.61	2,362	17.12	3.80	3.36	5.89
16	3,197	13.3	2.99	1,303	11.95	2.64	2.64	2.94
17	3,821	18.6	4.25	1,517	17.24	3.88	3.52	3.80
18	1,337	7.6	1.74	437	6.24	1.43	1.24	1.50
19=	2,818	18.8	4.27	878	15.14	3.66	3.54	4.40

Rabbit No. 334 - Infected

Day	Total ^{59}Fe Faecal Activity	^{59}Fe Blood Clearance (ml/24hrs)	^{59}Fe R.B.C. Clearance (ml/24hrs)	Total ^{51}Cr Faecal Activity	^{51}Cr Blood Clearance (ml/24hrs)	^{51}Cr R.B.C. Clearance (ml/24hrs)	Haemoglobin Fe/24hrs Lost into gut (mg)	Haemoglobin Fe/24hrs Excreted (mg)
1	1,409							
2	12,298	4.6	1.38					1.15
3	19,200	8.2	2.51					2.16
4	29,560	14.8	4.45					3.91
5	15,344	9.0	2.65	5,439				2.55
6	17,539	11.9	3.51	22,680	8.90	2.64	2.35	3.14
7	10,017	8.0	2.33	20,398	9.74	2.85	2.52	2.07
8	9,149	8.6	2.47	20,783	12.03	3.55	4.09	2.93
9	10,635	11.6	3.30	15,162	10.79	3.13	3.67	3.95
10	6,801	8.5	2.45	12,336	10.66	3.09	3.32	2.65
11	8,473	12.5	3.53	12,337	12.85	3.72	3.69	3.59
12	9,127	15.7	4.45	13,218	16.71	4.86	4.58	4.30
13	6,498	13.0	3.61	8,869	13.77	3.94	4.84	4.57
14	2,660	6.2	1.71	4,890	9.28	2.62	3.08	2.06
15	6,040	16.3	4.47	5,317	12.11	3.51	3.80	5.11
16	2,264	7.1	1.97	2,684	7.46	2.13	2.22	2.11
17	3,440	12.5	3.44	2,945	10.05	2.80	2.95	3.67
18	2,958	12.3	3.42	2,088	8.59	2.46	2.25	3.22
19	3,038	14.8	4.11	1,778	8.93	2.54	2.26	3.74

Rabbit No. 344 - Control

Day	Total ^{59}Fe Faecal Activity	^{59}Fe Blood Clearance (ml/24hrs)	^{59}Fe R.B.C. Clearance (ml/24hrs)	Total ^{51}Cr Faecal Activity	^{51}Cr Blood Clearance (ml/24hrs)	^{51}Cr R.B.C. Clearance (ml/24hrs)	Haemoglobin Fe/24hrs Lost into gut (mg)	Haemoglobin Fe/24hrs Excreted (mg)
1	1,099							
2	1,400	0.7	0.23					0.26
3	1,190	0.6	0.20					0.22
4	1,290	0.6	0.22					0.23
5	1,441	0.7	0.25	231				0.32
6	1,244	0.6	0.22	316	0.11	0.04	0.04	0.23
7	1,072	0.5	0.20	310	0.11	0.04	0.04	0.19
8	920	0.5	0.17	359	0.14	0.05	0.06	0.21
9	837	0.4	0.16	144	0.06	0.02	0.03	0.18
10	974	0.5	0.19	246	0.11	0.04	0.05	0.22
11	530	0.3	0.11	190	0.09	0.03	0.04	0.13
12	445	0.2	0.09	233	0.11	0.04	0.05	0.09
13	374	0.2	0.08	198	0.10	0.04	0.04	0.08
14	270	0.1	0.06	270	0.15	0.06	0.07	0.05
15	517	0.3	0.11	172	0.10	0.04	0.05	0.14
16	608	0.3	0.14	125	0.08	0.03	0.04	0.14
17	340	0.2	0.08	108	0.07	0.03	0.03	0.10
18	249	0.1	0.06	99	0.07	0.03	0.03	0.05
19	244	0.1	0.06	109	0.08	0.03	0.04	0.04

Rabbit No. 179 - Control

Day	Total ^{59}Fe Faecal Activity	^{59}Fe Blood Clearance (ml/24hrs)	^{59}Fe R.B.C. Clearance (ml/24hrs)	Total ^{51}Cr Faecal Activity	^{51}Cr Blood Clearance (ml/24hrs)	^{51}Cr R.B.C. Clearance (ml/24hrs)	Haemoglobin/24hrs Lost into gut (mg)	Haemoglobin/24hrs Excreted (mg)
1	700							
2	738	0.4	0.14					0.15
3	806	0.5	0.16					0.19
4	2,018	1.2	0.40					0.40
5	1,756	1.1	0.36	109				0.45
6	1,773	1.1	0.37	335	0.13	0.05	0.05	0.43
7	1,155	0.7	0.25	319	0.13	0.05	0.05	0.27
8	1,132	0.7	0.25	414	0.19	0.07	0.07	0.25
9	817	0.5	0.18	112	0.06	0.02	0.03	0.23
10	573	0.4	0.13	159	0.08	0.03	0.04	0.18
11	340	0.2	0.08	116	0.07	0.02	0.03	0.08
12	451	0.3	0.11	189	0.11	0.04	0.04	0.12
13	343	0.2	0.08	123	0.08	0.03	0.03	0.09
14	265	0.2	0.07	194	0.13	0.05	0.06	0.09
15	259	0.2	0.07	81	0.06	0.02	0.03	0.09
16	309	0.2	0.08	182	0.14	0.05	0.06	0.09
17	326	0.2	0.09	185	0.16	0.06	0.08	0.09
18	252	0.2	0.07	112	0.10	0.04	0.04	0.08
19	254	0.2	0.07	103	0.10	0.04	0.04	0.08

Rabbit No. 342 - Control

Day	Total ^{59}Fe Faecal Activity	^{59}Fe Blood Clearance (ml/24hrs)	^{59}Fe R.B.C. Clearance (ml/24hrs)	Total ^{51}Cr Faecal Activity	^{51}Cr Blood Clearance (ml/24hrs)	^{51}Cr R.B.C. Clearance (ml/24hrs)	Haemoglobin Fe/24hrs Lost into gut (mg)	Haemoglobin Fe/24hrs Excreted (mg)
1	1,872							
2	2,286	1.3	0.44					0.50
3	4,589	2.5	0.88					0.96
4	4,144	2.3	0.81					0.87
5	3,016	1.7	0.60	208				0.65
6	2,912	1.7	0.58	432	0.19	0.07	0.07	0.62
7	820	0.5	0.17	265	0.12	0.04	0.04	0.17
8	1,334	0.8	0.28	490	0.24	0.09	0.09	0.30
9	1,366	0.8	0.29	207	0.11	0.04	0.05	0.34
10	884	0.5	0.19	172	0.09	0.03	0.04	0.22
11	638	0.4	0.15	214	0.13	0.04	0.05	0.17
12	670	0.4	0.15	188	0.12	0.04	0.05	0.17
13	380	0.2	0.08	80	0.05	0.02	0.02	0.09
14	404	0.3	0.09	188	0.13	0.05	0.06	0.13
15	477	0.3	0.11	108	0.08	0.03	0.03	0.14
16	192	0.1	0.04	66	0.05	0.02	0.02	0.04
17	281	0.1	0.07	57	0.05	0.02	0.02	0.04
18	252	0.2	0.06	98	0.08	0.03	0.03	0.04
19	225	0.1	0.05	105	0.09	0.03	0.04	0.08

Rabbit No. 201 - control

Day	Total ^{59}Fe Faecal Activity	^{59}Fe Blood Clearance (ml/24hrs)	^{59}Fe R.B.C. Clearance (ml/24hrs)	Total ^{51}Cr Faecal Activity	^{51}Cr Blood Clearance (ml/24hrs)	^{51}Cr R.B.C. Clearance (ml/24hrs)	Haemoglobin/24hrs Lost into gut (mg)	Haemoglobin/24hrs Excreted (mg)
1	669							
2	1,100	0.52	0.16					0.21
3	1,696	0.83	0.26					0.35
4	1,685	0.84	0.26					0.29
5	1,987	1.02	0.32	193				0.38
6	1,892	0.99	0.32	103	0.04	0.01	0.02	0.38
7	1,638	0.88	0.28	390	0.17	0.06	0.07	0.35
8	1,246	0.68	0.22	402	0.19	0.07	0.08	0.28
9	826	0.46	0.15	71	0.04	0.01	0.01	0.17
10	528	0.30	0.09	86	0.05	0.02	0.02	0.12
11	418	0.25	0.08	76	0.04	0.02	0.02	0.12
12	475	0.28	0.09	112	0.07	0.03	0.03	0.12
13	490	0.30	0.10	125	0.08	0.03	0.03	0.12
14	252	0.16	0.05	180	0.13	0.05	0.05	0.06
15	304	0.19	0.07	39	0.03	0.01	0.01	0.09
16	317	0.21	0.07	110	0.09	0.03	0.04	0.09
17	256	0.17	0.06	122	0.11	0.05	0.04	0.06
18	192	0.13	0.05	80	0.07	0.03	0.03	0.05
19	283	0.20	0.07	83	0.08	0.03	0.03	0.08

^{59}Fe / ^{51}Cr R.B.C. Turnover Studies in Normal and Fluke-Infected Rabbits

Faecal and Urinary Excretion of ^{59}Fe and ^{51}Cr

	Rabbit No.	^{51}Cr Activity: Cumulative Urine Activity as % Injected (15 days)	^{51}Cr Activity: Cumulative Faecal Activity as % Injected (15 days)	^{59}Fe Activity: Cumulative Faecal Activity as % Injected (19 days)
INFECTED	316	13.32	55.86	63.48
	BL	20.68	40.41	62.57
	340	13.96	55.09	63.92
	334	11.24	56.29	63.18
	335	11.56	44.00	62.83
	339	13.84	35.85	52.99
	Mean	14.10	47.92	61.50
	S.D.	3.42	8.97	4.19
CONTROL	344	10.71	0.98	6.26
	179	21.46	1.03	6.33
	201	22.45	0.77	10.41
	342	21.89	0.97	5.37
	Mean	19.13	0.94	7.09
	S.D.	5.62	0.11	2.25
	P	>0.10	<0.001	<0.001

APPENDIX C

SIMULTANEOUS MEASUREMENT OF THE LOSS OF RED CELLS AND PLASMA INTO THE GUT OF NORMAL AND FLUKE-INFECTED RABBITS AND THE EFFECT OF ANTHELMINTIC TREATMENT ON THE MAGNITUDE OF THESE LOSSES

Effect of Anthelmintic Treatment on the Turnover of ^{51}Cr - labelled Erythrocytes and ^{95}Nb - labelled Albumin in Normal and Fluke-infected Rabbits

Daily Faecal "Clearances" of Plasma, Blood and Red Cells

RABBIT NO. 020 - INFECTED

Day of Expt.	^{51}Cr faecal Activity	^{95}Nb faecal Activity	Faecal Clearance(ml/day)		
			Blood	Red Cells	Plasma
2	--8898-	-479----	--	---	---
3	8098	479	13.92	4.89	29.33
4	3546	240	10.84	2.66	16.75
5	3759	252	14.86	3.55	19.89
6	2703	228	13.71	3.26	20.36
7	3331	265	21.74	4.99	27.04
8	2213	185	19.63	4.18	21.35
9	1305	215	14.08	3.15	28.04
10	1495	258	20.52	4.48	38.13
TREATED		Mean	16.16	3.90	25.11
11	233	31	4.21	0.88	5.26
12	73	35	1.36	0.34	6.69
13	750	174	16.64	4.19	37.29
14	596	180	12.73	3.55	38.57
15	239	83	4.87	1.53	11.86
16	147	49	4.24	1.01	8.17
17	105	41	3.42	0.79	6.83
18	39	18	1.10	0.33	2.16
19	Not Sig.	Not Sig.	--	--	---

Effect of Anthelmintic Treatment on the Turnover of ^{51}Cr - labelled Erythrocytes and ^{95}Nb - labelled Albumin in Normal and Fluke-infected Rabbits

Daily Faecal "Clearances" of Plasma, Blood and Red Cells

RABBIT NO. - 182 - INFECTED

Day of Expt.	^{51}Cr faecal Activity	^{95}Nb faecal Activity	Faecal Clearance (ml/day)		
			Blood	Red Cells	Plasma
2	2286	345	5.32	1.37	19.22
3	2691	286	6.07	1.85	16.53
4	2491	359	6.45	1.94	17.27
5	1560	180	4.53	1.38	13.85
6	1599	258	5.36	1.62	22.76
7	1480	168	5.52	1.71	17.03
8	1524	171	6.52	2.00	19.81
9	900	162	4.47	1.37	21.60
10	1373	135	7.76	2.33	20.88
TREATED		Mean	5.78	1.73	18.77
11	632	86	4.23	1.24	15.45
12	148	66	1.16	0.33	13.56
13	124	101	0.70	0.26	11.43
14	52	69	0.43	0.13	10.35
15	126	47	1.02	0.33	13.43
16	103	16	0.91	0.30	5.65
17	50	24	0.56	0.17	6.78
18	28	18	0.33	0.12	4.82

Effect of Anthelmintic Treatment on the Turnover of ^{51}Cr - labelled Erythrocytes and ^{95}Nb - labelled Albumin in Normal and Fluke-infected Rabbits

Daily Faecal "Clearances" of Plasma, Blood and Red Cells

RABBIT NO. 712 - INFECTED

Day of Expt.	^{51}Cr faecal Activity	^{95}Nb faecal Activity	Faecal Clearance (ml/day)		
			Blood	Red Cells	Plasma
2	2316	648	4.92	1.35	34.72
3	2318	504	5.32	1.58	35.16
4	1877	388	5.16	1.50	33.26
5	1648	369	5.53	1.53	34.81
6	1695	372	6.71	1.86	38.48
7	1109	267	5.27	1.44	30.82
8	1189	262	6.76	1.79	33.45
9	763	301	5.12	1.33	42.39
10	1025	308	8.33	2.12	47.88
TREATED		Mean	5.90	1.61	36.78
11	374	114	3.54	0.91	19.00
12	377	193	4.23	1.05	22.71
13	86	118	0.97	0.28	15.39
14	107	132	1.42	0.41	21.41
15	71	71	1.14	0.32	11.21
16	52	49	1.06	0.28	9.08
17	71	50	1.65	0.27	13.64
18	32	26	0.82	0.15	8.38

At P.M. 15 Dead Adult Flukes.

Effect of Anthelmintic Treatment on the Turnover of ^{51}Cr - labelled Erythrocytes and ^{95}Nb - labelled Albumin in Normal and Fluke-infected Rabbits.

RABBIT NO. - 332 - INFECTED - Daily Blood "Clearances"

Day of Expt.	^{51}Cr faecal Activity	Blood Clearance(ml/day)
2	4263	8.6
3	4992	10.8
4	3290	9.0
5	3667	13.0
6	3731	16.6
	Mean	11.5

At P.M. 33 Live Adult Flukes.

RABBIT NO. 314 - INFECTED - Daily Blood "Clearances"

2	5926	12.8
3	7044	18.1
4	4756	15.2
5	3410	13.6
6	2960	14.9
7	2856	13.2
8	2128	16.6
9	1299	13.1
10	1487	18.2
	Mean	15.6

Effect of Anthelmintic Treatment on the Turnover of ^{51}Cr - labelled Erythrocytes and ^{95}Nb - labelled Albumin in Normal and Fluke-infected Rabbits

Daily Faecal "Clearances" of Plasma, Blood and Red Cells

RABBIT NO. 384 - CONTROL

Day of Expt.	^{51}Cr faecal Activity	^{95}Nb faecal Activity	Faecal Clearance (ml/day)		
			Blood	Red Cells	Plasma
2	92	64	0.06	0.03	4.00
3	29	84	0.09	0.02	9.33
4	39	49	0.06	0.03	6.68
5	24	76	0.10	0.02	11.69
6	42	125	0.21	0.04	21.43
7	70	45	0.13	0.07	8.59
8	31	47	0.17	0.03	10.00
9	48	59	0.22	0.06	14.05
10	57	28	0.30	0.08	7.43
TREATED		Mean	0.13	0.04	10.4 (Enteritis)
11	10	46	0.04	0.01	13.80
12	17	43	0.08	0.03	12.06
13	47	34	0.23	0.08	14.78
14	15	27	0.09	0.03	11.74
		Mean	0.11	0.04	13.1

Effect of Anthelmintic Treatment on the Turnover of ^{51}Cr - labelled Red Cells and ^{95}Nb - labelled Albumin in Normal and Fluke-infected Rabbits

Daily Faecal "Clearances" of Plasma, Blood and Red Cells

RABBIT NO. 027 - CONTROL

Day of Expt.	^{51}Cr faecal Activity	^{95}Nb faecal Activity	Faecal Clearance(ml/day)		
			Blood	Red Cells	Plasma
2	27	41	0.05	0.02	2.28
3	35	35	0.07	0.02	3.04
4	25	25	0.05	0.02	2.98
5	25	27	0.06	0.02	3.95
6	29	31	0.07	0.02	5.08
7	20	27	0.05	0.02	4.92
8	27	17	0.08	0.03	8.32
9	26	28	0.08	0.03	6.77
10	18	20	0.06	0.02	5.13
TREATED		Mean	0.06	0.02	4.20
11	22	31	0.08	0.03	9.03
12	18	20	0.07	0.02	5.22
13	14	16	0.06	0.02	3.65
14	4	21	0.02	0.01	4.22
15	20	12	0.10	0.04	3.67
		Mean	0.07	0.02	5.20

Effect of Anthelmintic Treatment on the Turnover of ^{51}Cr - labelled Erythrocytes and ^{95}Nb - labelled Albumin in Normal and Fluke-infected Rabbits

Daily Faecal Clearances of Plasma, Blood and Red Cells

RABBIT NO. - X = CONTROL

Day of Expt.	^{51}Cr faecal Activity	^{95}Nb faecal Activity	Faecal Clearance(ml/day)	
			Blood	Red Cells
2	117	Activity too	0.24	0.08
3	32	low to measure	0.06	0.02
4	45		0.09	0.03
5	52		0.12	0.04
6	172		0.43	0.15
7	32		0.08	0.03
8	19		0.05	0.02
9	16		0.03	0.02
10	10		0.03	0.02
TREATED		Mean	0.13	0.04
11	11		0.04	0.01
12	11		0.05	0.02
13	36		0.23	0.09
14	26		0.23	0.09
15	31		0.44	0.17
16	12		0.21	0.08
17	16		0.30	0.13
18	6		0.14	0.08
		Mean	0.20	0.08

Effect of Anthelmintic Treatment on the Turnover of ^{51}Cr - labelled Erythrocytes and ^{95}Nb - labelled Albumin in Normal and Fluke-infected Rabbits

RABBIT NO. - 055 - CONTROL - Daily Plasma "Clearances"

Day of Expt.	^{95}Nb faecal Activity	Plasma Clearance (ml/day)
2	28	2.15
3	24	2.53
4	53	6.00
5	65	7.96
6	38	4.96
7	32	4.53
8	38	5.76
9	11	1.78
10	24	2.24
	Mean	4.21

RABBIT NO. - 057 - CONTROL - Daily plasma "Clearances"

2	71	5.07
3	37	4.74
4	53	8.60
5	36	5.08
6	24	4.80
7	36	7.94
8	31	7.50
9	16	4.29
10	17	5.00
	Mean	5.89

EFFECT OF ANTHELMINTIC TREATMENT ON THE PASSAGE OF ^{131}I-LABELLED P.V.P. INTO THE GASTROINTESTINAL TRACT OF NORMAL AND FLUKE-INFECTED RABBITS

Effect of Anthelmintic Treatment on the Passage of ^{131}I-labelled P.V.P. into the Gastro-intestinal Tract of Normal and Fluke-infected Rabbits

Rabbit No. 110 Control

Day of Expt.	Total Faecal Activity	Cumulative Faecal Activity	Cumulative Faecal Activity as % Inj. dose	Plasma Clearance (mls)
0	858			
1	1,080	1.938	0.33	0.8
2	369	2,307	0.40	0.3
3	606	2,913	0.50	0.7
4	976	3,889	0.66	1.3
5	1,160	3,049	0.86	1.9
6	690	5,739	0.97	1.3
Mean				1.1
Treated				
7	842	6,581	1.1	1.8
8	580	7,161	1.2	1.5
9	546	7,707	1.3	1.5
10	890	8,597	1.5	2.8
11	857	9,454	1.6	3.1
12	585	10,039	1.7	2.4
13	364	10,403	1.8	1.7

Mean calculated from Days 2 - 6.

Effect of Anthelmintic Treatment on the Passage of ^{131}I-labelled P.V.P. into the Gastro-intestinal Tract of Normal and Fluke-infected Rabbits

Rabbit No. 039 - Control

Day of Expt.	Total Faecal Activity	Cumulative Faecal Activity	Cumulative Faecal Activity as % Inj.dose	Plasma Clearance (mls)
0	1,1157			
1	1,520	2,677	0.4	1.0
2	1,481	4,158	0.7	1.2
3	1,272	5,430	0.9	1.2
4	1,622	7,052	1.1	2.0
5	963	8,015	1.3	1.4
6	665	8,680	1.4	1.2
Mean				1.4
Treated				
7=	378	9,058	1.5	0.8
8	574	9,632	1.6	1.5
9	1,210	10,842	1.8	3.9
10	658	11,500	1.9	2.6
11	563	12,063	2.0	2.4
12	276	12,339	2.0	1.3
13	293	12,632	2.1	1.6

Effect of Anthelmintic Treatment on the Passage of ^{131}I-labelled P.V.P. into the Gastro-intestinal Tract of Normal and Fluke-infected Rabbits

Rabbit No.203 - Control

Day of Expt.	Total Faecal Activity	Cumulative Faecal Activity	Cumulative Faecal Activity as % Inj. dose	Plasma Clearance (mls)
0	202			
1	680	882	0.15	0.5
2	930	1,812	0.3	0.8
3	1,899	3,711	0.6	1.9
4	1,281	4,992	0.85	1.6
5	1,548	6,540	1.1	2.4
6	725	7,265	1.2	1.3
Mean				1.6
Treated				
7	262	7,527	1.3	0.6
8	933	8,460	1.4	2.2
9	1,900	10,360	1.8	5.2
10	1,232	11,592	2.0	3.7
11	812	12,404	2.1	3.0
12	461	12,865	2.2	2.0
13	140	13,005	2.2	0.7

Effect of Anthelmintic Treatment on the Passage of ^{131}I-labelled P.V.P. into the Gastro-intestinal Tract of Normal and Fluke-infected Rabbits

Rabbit No. 286 - Infected

Day of Expt.	Total Faecal Activity (counts/sec)	Accumulative Faecal Activity (counts/sec)	Cumulative Faecal Activity as % Inj.dose	Plasma Clearance (mls)
0	12,499			
1	11,721	24,220	3.9	9.0
2	12,707	36,927	6.0	14.0
3	13,718	50,690	8.3	22.0
4	8,041	58,731	9.6	18.5
5	4,256	62,987	10.3	14.0
6	2,963	65,950	10.8	13.9
Mean				16.5
Treated				
7	1,444	67,394	11.1	9.7
8	108	67,502	11.1	0.9
9	268	67,770	11.1	2.4
10	1,164	68,934	11.3	12.25
11	424	69,358	11.4	4.9
12	313	69,671	11.4	4.0
13	156	69,827	11.5	2.0

Effect of Anthelmintic Treatment on the Passage of ^{131}I-labelled P.V.P. into the Gastro-intestinal Tract of Normal and Fluke-infected Rabbits

Rabbit No.258 - Infected

Day of Expt.	Total Faecal Activity	Cumulative Faecal Activity	Cumulative Faecal Activity as % Inj.dose	Plasma Clearance (mls)
0	6,415			
1	11,915	18,330	3.0	8.4
2	10,123	28,453	4.6	9.1
3	9,168	37,621	6.1	10.6
4	7,404	45,052	7.3	10.7
5	4,089	49,114	8.0	7.7
6	3,761	52,875	8.6	9.1
Mean				9.4
Treated				
7	1,565	54,440	8.8	4.8
8	1,450	55,890	9.1	5.9
9	1,741	57,631	9.4	8.7
10	968	58,500	9.5	5.4
11	468	58,968	9.6	3.1
12	328	59,296	9.6	1.5
13	185	59,481	9.7	1.5

Effect of Anthelmintic Treatment on thePassage of ^{131}I-labelled P.V.P. into the Gastro-intestinal Tract of Normal and Fluke-infected Rabbits

Rabbit No. 263 - Infected

Day of Expt.	Total Faecal Activity	Cumulative Faecal Activity	Cumulative Faecal Activity as% Inj.dose	Plasma Clearance (mls)
0	8,507			
1	13,244	21,751	3.7	9.3
2	11,412	33,163	5.7	11.0
3	8,235	41,398	7.0	10.7
4	8,333	49,731	8.5	14.8
5	3,872	53,603	9.2	9.4
6	2,116	55,719	9.5	6.9
Mean				10.4
Treated				
7	2,797	58,516	10.0	12.3
8	283	58,799	10.0	
9	Died			

EFFECT OF ANTHELMINTIC TREATMENT ON THE PASSAGE OF ^{51}Cr INTO THE GASTROINTESTINAL TRACT OF NORMAL AND FLUKE-INFECTED RABBITS

Effect of Anthelmintic Treatment on the Turnover of ^{51}Cr-labelled Erythrocytes in Normal and Fluke-infected Rabbits

Rabbit No. 423 - Control

Day of Expt.	Faecal Activity	Cumulative Activity	Cumulative as % Inj.	Whole Blood (ml/24 hrs)	R.B.C. (ml/24hrs)
0	26				
1	76	102	0.05	0.04	0.02
2	86	188	0.09	0.05	0.02
3	152	340	0.16	0.09	0.04
4	56	396	0.18	0.04	0.01
5	48	444	0.21	0.03	0.01
6	132	576	0.27	0.09	0.04
7	56	632	0.29	0.04	0.02
8	50	682	0.32	0.04	0.02
9	45	727	0.34	0.04	0.02
10	61	788	0.37	0.06	0.02
11	97	885	0.41	0.09	0.04
12	143	1,028	0.48	0.14	0.05
Treated					
13	56	1,084	0.50	0.06	0.02
14	82	1,166	0.54	0.09	0.03
15	169	1,335	0.62	0.20	0.07
16	253	1,588	0.74	0.31	0.11
17	149	1,737	0.81	0.19	0.06
18	140	1,877	0.87	0.19	0.06
19	109	1,986	0.92	0.15	0.05
20	143	2,129	0.99	0.20	0.06
21	91	2,220	1.03	0.14	0.04
22	65	2,285	1.06	0.10	0.04
23	160	2,445	1.14	0.26	0.08
24	71	2,516	1.17	0.12	0.04
25	39	2,555	1.19	0.07	0.02
26	42	2,597	1.21	0.08	0.02
			Mean pre-treatment	0.06	0.03
			Mean Post-treatment	0.15	0.05

Effect of Anthelmintic Treatment on the Turnover of ^{51}Cr-labelled Erythrocytes in Normal and Fluke-infected Rabbits

Rabbit No. 424 - Control

Day of Expt.	Faecal Activity	Cumulative Activity	Cumulative as % Inj.	Whole Blood (ml/24hrs)	R.B.C. (ml/24hrs)
0	28				
1	93	121	0.06	0.04	0.02
2	90	211	0.10	0.04	0.02
3	73	284	0.13	0.04	0.02
4	27	311	0.15	0.02	0.01
5	26	337	0.16	0.01	0.01
6	12	349	0.16	0.01	0.01
7	112	461	0.22	0.07	0.02
8	111	572	0.27	0.07	0.02
9	124	696	0.33	0.08	0.03
10	106	802	0.38	0.07	0.02
11	149	951	0.45	0.11	0.03
12	89	1,040	0.49	0.06	0.02
Treated					
13	74	1,114	0.52	0.06	0.02
14	114	1,228	0.58	0.09	0.03
15	176	1,404	0.66	0.15	0.05
16	28	1,432	0.67	0.03	0.01
17	-	1,432	0.67	-	-
18	33	1,465	0.69	0.03	0.01
19	55	1,520	0.71	0.06	0.02
20	53	1,573	0.74	0.06	0.02
21	126	1,699	0.80	0.14	0.04
22	107	1,806	0.85	0.12	0.04
23	86	1,892	0.89	0.10	0.03
24	47	1,939	0.91	0.06	0.02
25	43	1,982	0.93	0.06	0.02
26	48	2,030	0.95	0.07	0.02
		Mean Pre-treatment		0.06	0.02
		Mean Post-treatment		0.07	0.02

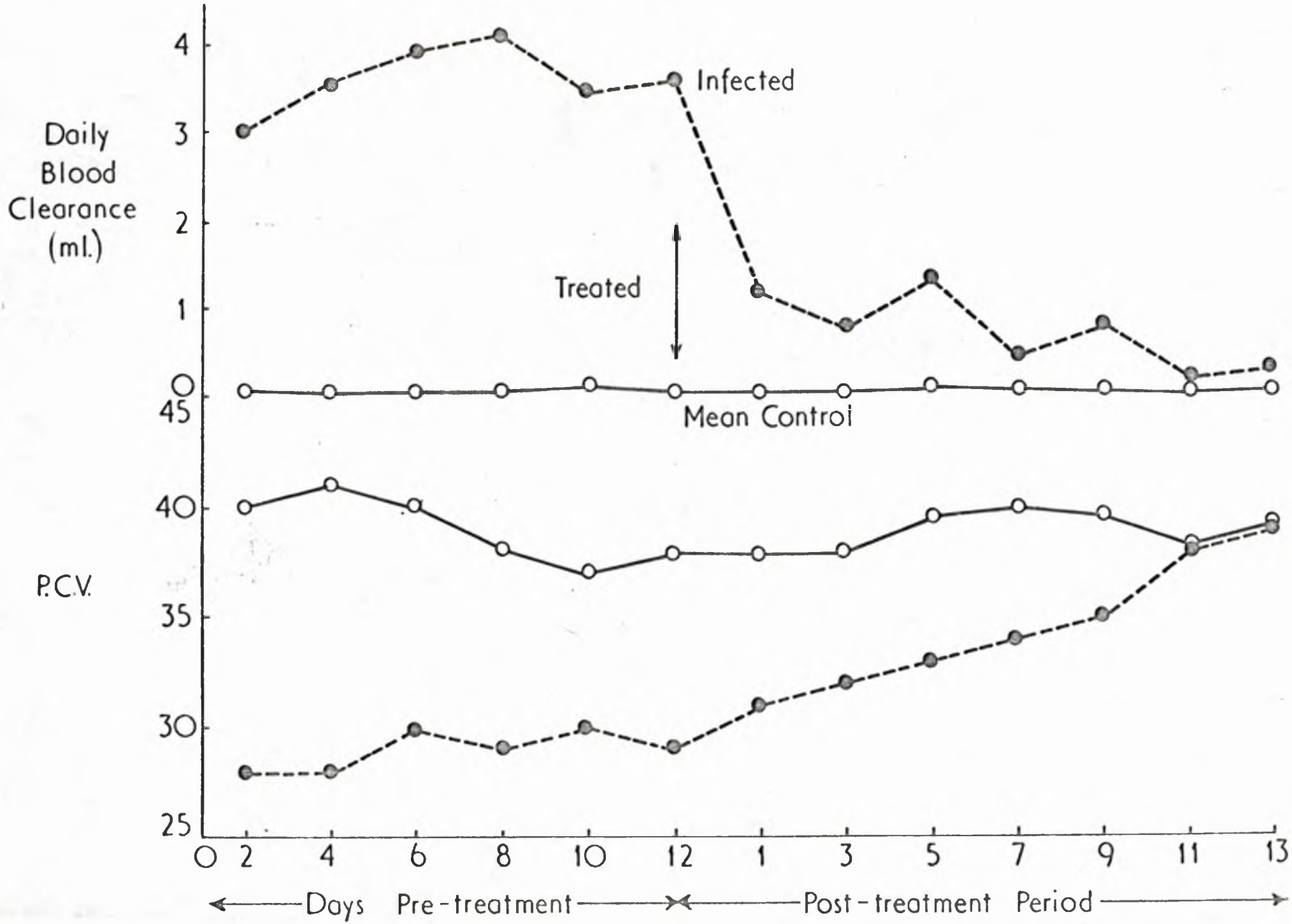
Effect of Anthelmintic Treatment onthe Turnover of ^{51}Cr-labelled Erythrocytes in Normal and Fluke-infected Rabbits

Rabbit No. 320 - Infected

Day of Expt.	Faecal Activity	Cumulative Activity	Cumulative as % Inj.	Whole Blood (ml/24hrs)	R.B.C. (ml/24hrs)
0					
1	154				
2	4,415	4,569	2.19	2.65	0.77
3	5,141	9,710	4.66	3.45	1.00
4	4,586	14,296	6.86	3.39	1.00
5	4,812	19,108	9.17	4.03	1.17
6	3,213	22,321	10.71	2.97	0.87
7	4,692	27,013	12.97	4.84	1.43
8	3,581	30,594	14.68	4.18	1.22
9	2,664	33,258	15.96	3.48	1.05
10	2,413	35,671	17.12	3.35	1.04
11	2,261	37,932	18.21	3.58	1.07
12	2,036	39,968	19.18	3.61	1.06
Treated					
13	594	40,562	19.47	1.20	0.35
14	139	40,701	19.54	0.29	0.09
15	361	41,062	19.71	0.82	0.26
16	1,177	42,239	20.27	2.89	0.93
17	517	42,765	20.52	1.35	0.45
18	248	43,004	20.64	0.71	0.23
19	158	43,162	20.72	0.48	0.17
20	230	43,392	20.83	0.76	0.26
21	237	43,629	20.94	0.84	0.30
22	148	43,777	21.01	0.56	0.20
23	49	43,826	21.04	0.20	0.07
24	119	43,945	21.09	0.52	0.19
25	72	44,017	21.13	0.34	0.13
26	64	44,081	21.16	0.32	0.13
			Mean Pre-treatment	3.59	1.06
			Mean Post-treatment	0.81	0.27

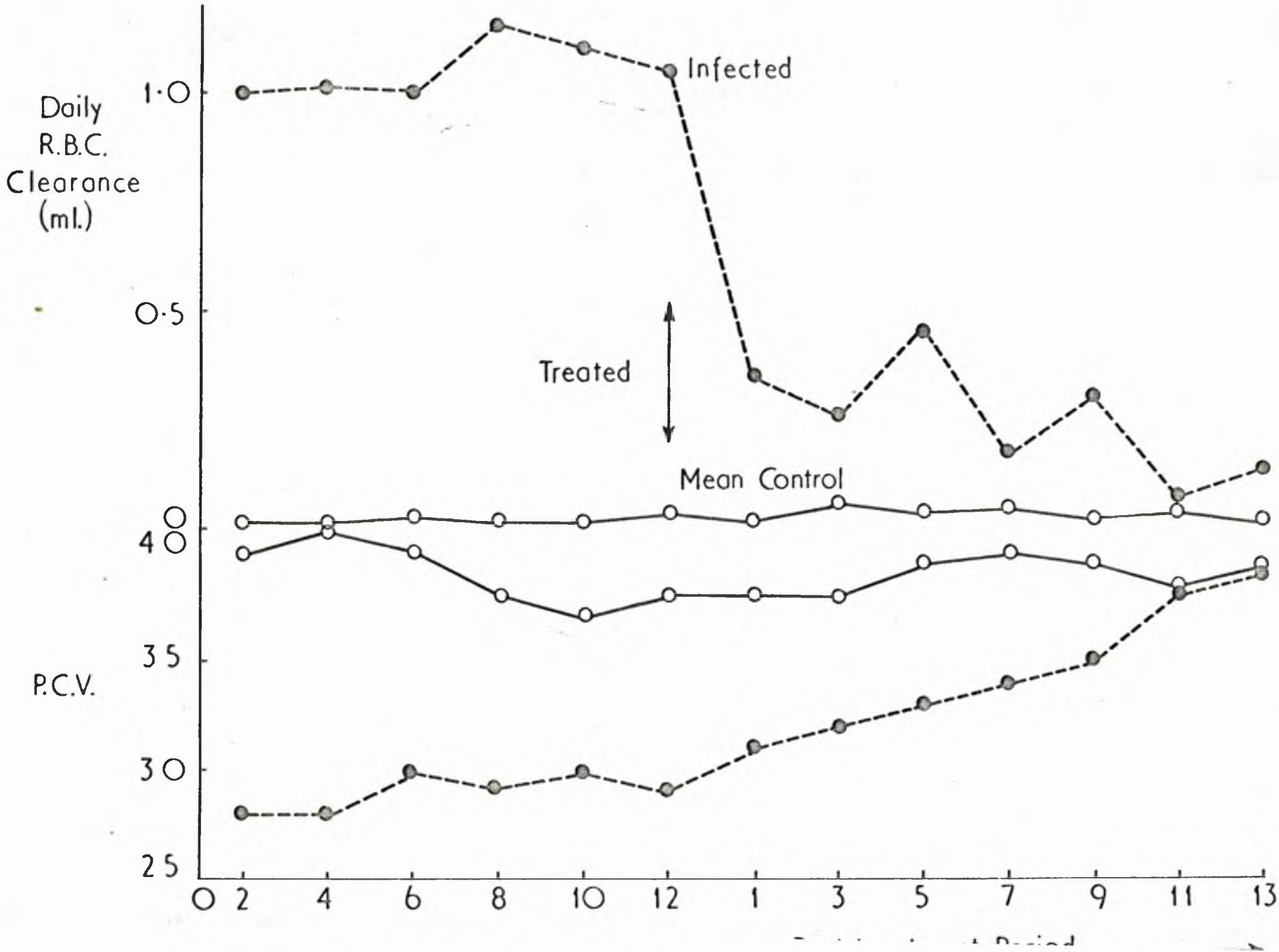
Effect of Anthelmintic treatment on turnover of ^{51}Cr-Labelled Erythrocytes Rabbit No. 320

P.C.V. and Blood Clearances Pre- and Post-treatment



Effect of Anthelmintic on the turnover of ^{51}Cr-Labelled Erythrocytes Rabbit No. 320

P.C.V. and R.B.C. Clearances Pre- and Post-treatment



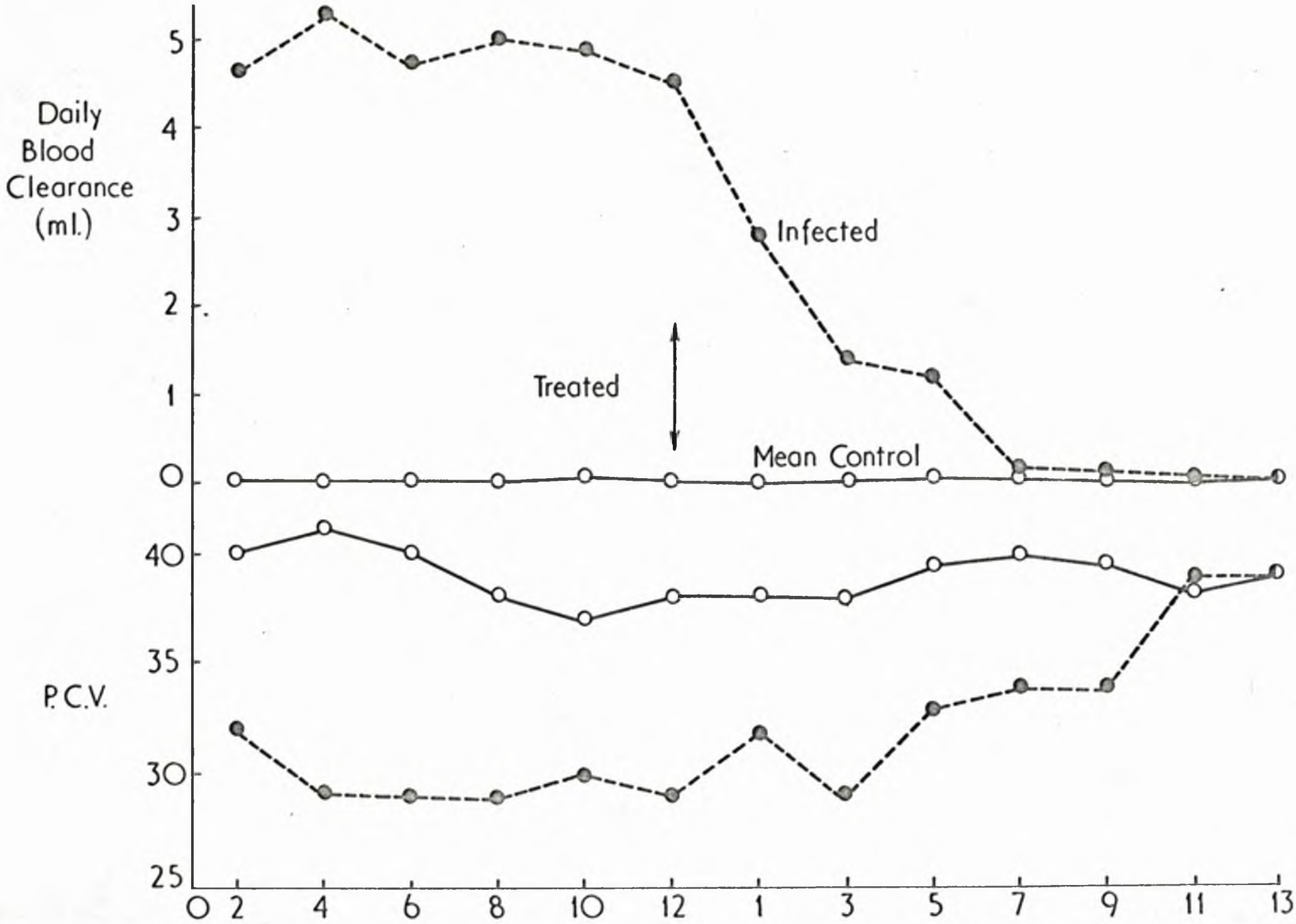
Effect of Anthelmintic Treatment on the Turnover of ^{51}Cr-labelled Erythrocytes in Normal and Fluke-infected Rabbits

Rabbit No. 346 - Infected

Day of Expt.	Faecal Activity	Cumulative Activity	Cumulative as % Inj.	Whole Blood (ml/24hrs)	R.B.C. (ml/24hrs)
0	70				
1	367	437	0.21	0.18	0.06
2	6,374	6,811	3.30	3.43	1.02
3	7,501	14,312	6.94	4.51	1.35
4	8,904	23,216	11.25	5.96	1.81
5	9,173	32,389	15.70	6.72	2.09
6	3,854	36,243	17.56	3.17	0.99
7	6,314	42,557	20.62	5.80	1.85
8	5,038	47,595	23.06	5.25	1.66
9	4,990	52,585	25.48	5.70	1.89
10	3,265	55,850	27.07	4.13	1.40
11	4,286	60,136	29.14	6.08	2.07
12	2,864	63,000	30.53	4.48	1.53
Treated					
13	1,566	64,566	31.29	2.82	0.89
14		64,566	31.29	-	-
15	333	64,899	31.45	1.47	0.22
16	445	65,344	31.67	0.93	0.33
17	637	65,981	31.97	1.42	0.51
18	333	66,314	32.14	0.80	0.29
19	190	66,504	32.23	0.49	0.18
20	140	66,644	32.30	0.39	0.14
21	131	66,775	32.36	0.38	0.14
22	64	66,839	32.39	0.20	0.08
23	94	66,933	32.44	0.31	0.12
24	42	66,975	32.46	0.15	0.06
25	43	67,015	32.48	0.17	0.06
26	40	67,055	32.50	0.18	0.06
			Mean Pre-treatment	5.02	1.61
			Mean Post-treatment	0.69	0.22

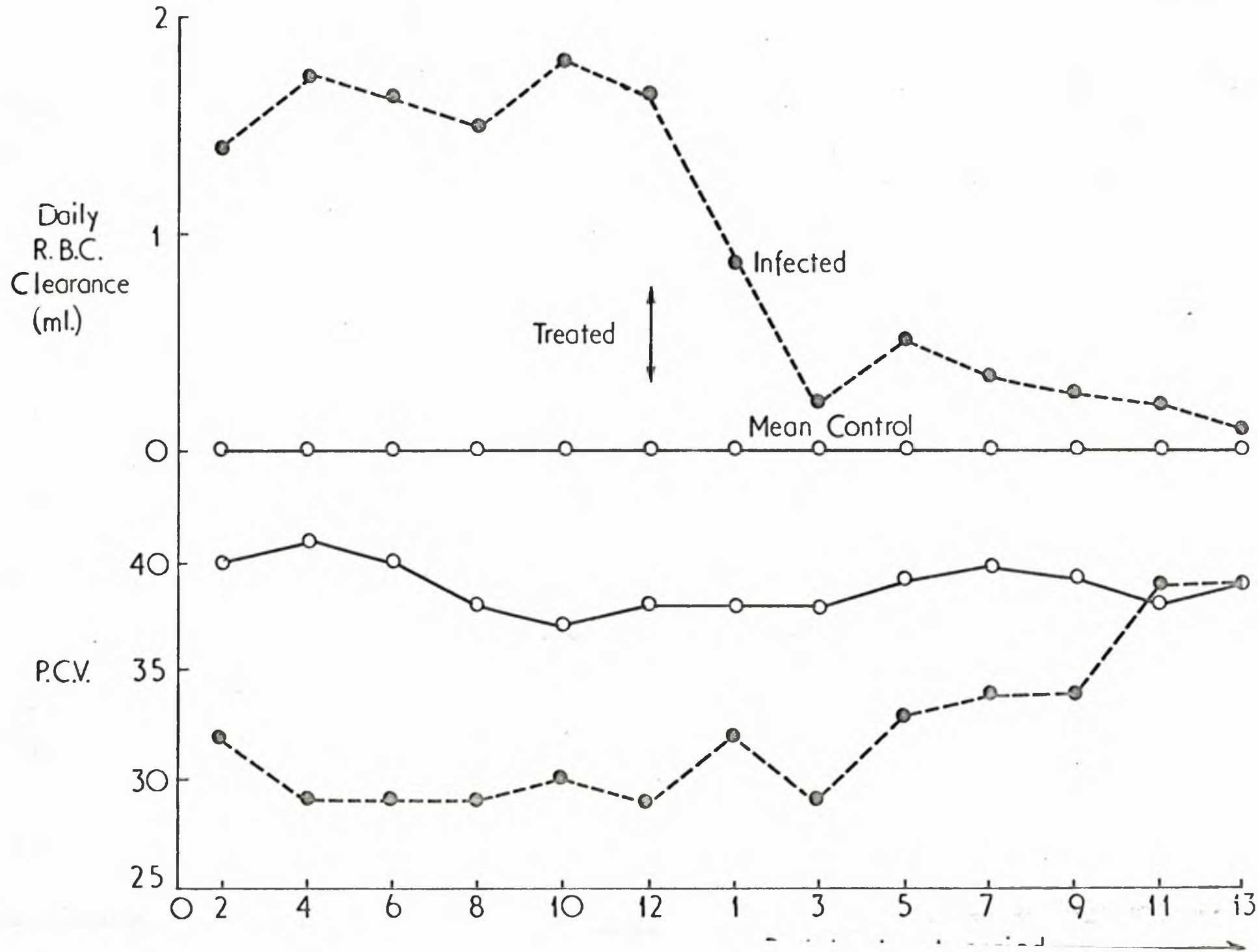
Effect of Anthelmintic Treatment on turnover of ^{51}Cr-Labelled Erythrocytes Rabbit No. 346

P.C.V. and Blood Clearances Pre- and Post-treatment



Effect of Anthelmintic Treatment on turnover of ^{51}Cr-Labelled Erythrocytes Rabbit No 346

P.C.V. and R.B.C. Clearances Pre-and Post-treatment



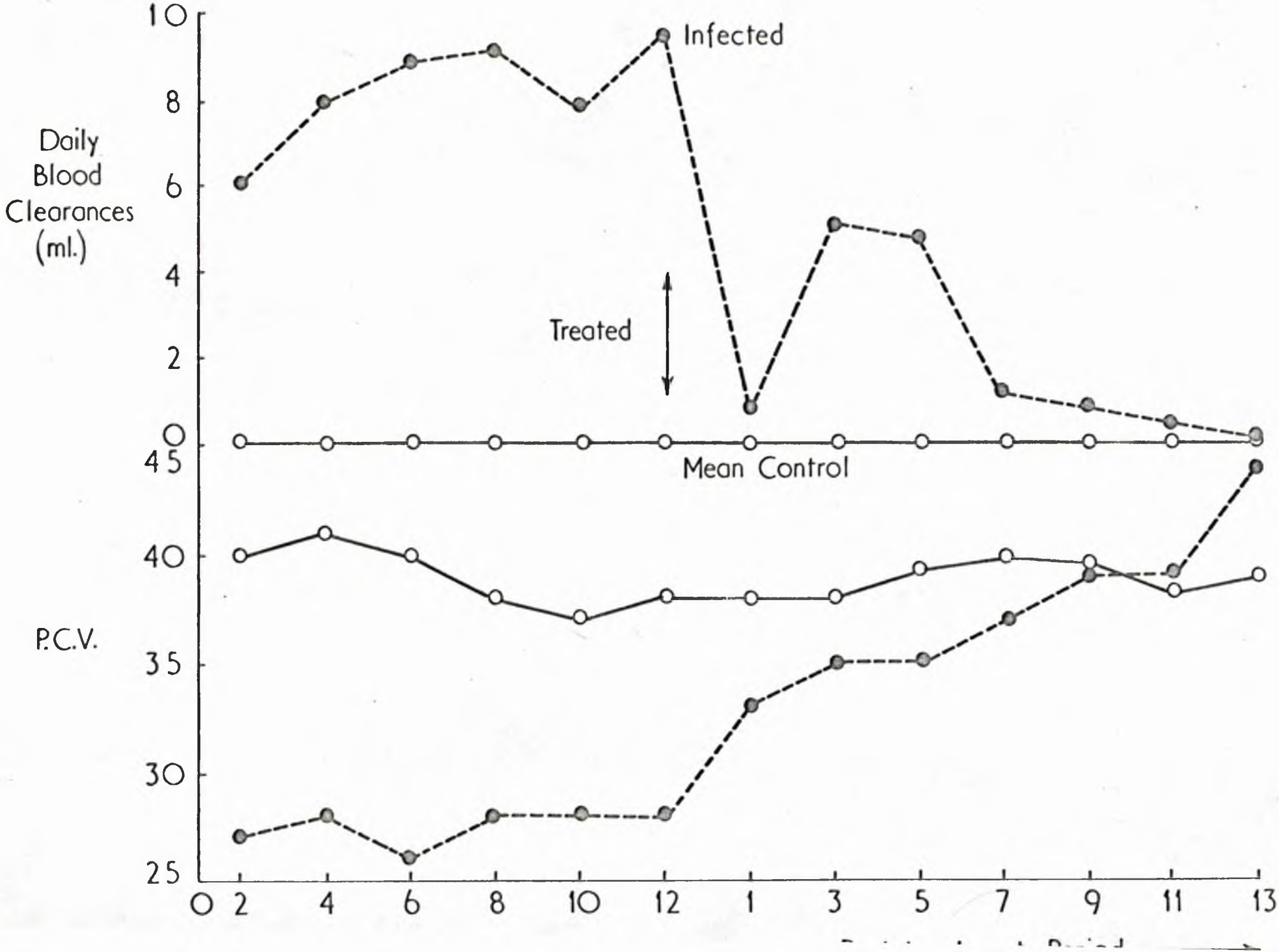
Effect of Anthelmintic Treatment on the Turnover of ^{51}Cr-labelled Erythrocytes in Normal and Fluke-infected Rabbits

Rabbit No. 438 - Infected

Day of Expt.	Faecal Activity	Cumulative Activity	Cumulative as % Inj.	Whole Blood (ml/24hrs)	R.B.C. (ml/24hours)
0	161				
1	593	754	0.40	0.28	0.08
2	11,660	12,414	6.57	6.42	1.74
3	8,676	21,090	11.16	5.63	1.53
4	10,389	31,679	16.76	8.03	2.17
5	11,638	43,317	22.92	10.18	2.83
6	7,372	50,689	26.82	7.60	2.10
7	7,207	57,896	30.63	8.79	2.40
8	5,791	63,687	33.70	8.32	2.25
9	5,602	69,289	36.66	9.38	2.61
10	4,935	74,224	39.27	9.93	2.62
11	5,296	79,520	42.08	2.52	3.34
12	3,571	83,091	43.96	9.57	2.60
Treated					
13	284	83,375	44.12	0.91	0.23
14	270	83,645	44.26	0.93	0.26
15	1,377	85,022	44.99	5.04	1.46
16	2,244	87,266	46.17	8.80	2.73
17	1,156	88,422	46.79	4.84	1.57
18	402	88,824	46.99	1.79	0.63
19	248	89,072	47.13	1.19	0.44
20	179	89,251	47.22	0.92	0.35
21	168	89,419	47.31	0.94	0.38
22	128	89,547	47.80	0.76	0.32
23	80	89,627	47.42	0.50	0.23
24	55	89,682	47.45	0.37	0.18
25	32	89,714	47.47	0.23	0.12
26	33	89,747	47.49	0.26	0.14
			Mean Pre-treatment	7.45	2.38
			Mean Post-treatment	1.96	0.65

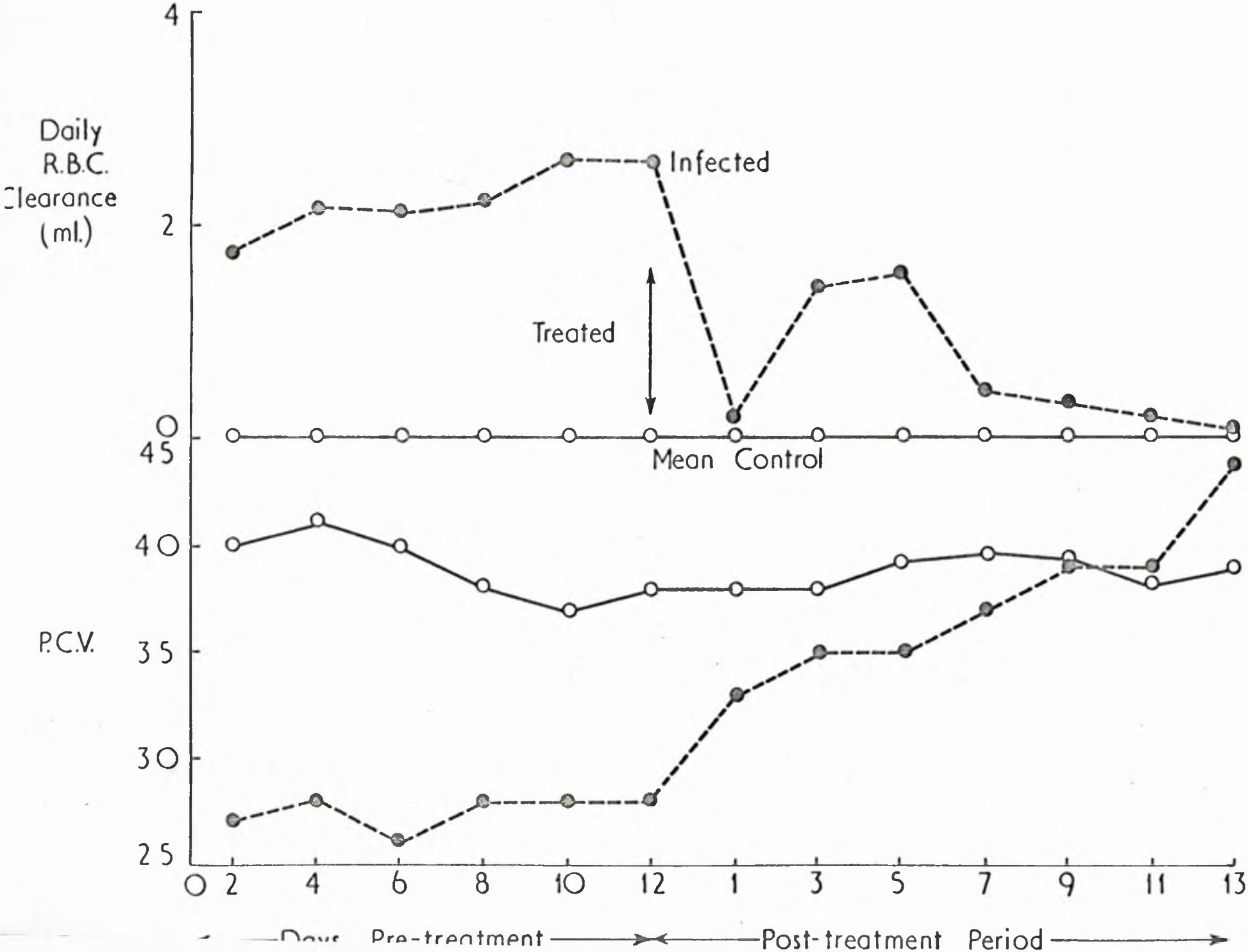
Effect of Anthelmintic treatment on the turnover of ^{51}Cr-Labelled Erythrocytes Rabbit No 438

P.C.V. and Blood Clearances Pre-and Post-Treatment



Effect of Anthelmintic on the turnover of ^{51}Cr-Labelled Erythrocytes Rabbit No. 438

P.C.V. and Blood Clearances Pre- and Post-treatment



APPENDIX D

A STUDY OF THE ONSET AND DEVELOPMENT OF THE ANAEMIA AND HYPOPROTEINAEMIA ASSOCIATED WITH CHRONIC FASCIOLIASIS

^{125}I-LABELLED ALBUMIN/ ^{51}Cr-LABELLED ERYTHROCYTES EXPERIMENTS

PHASE 1:- EXPERIMENTAL RESULTS

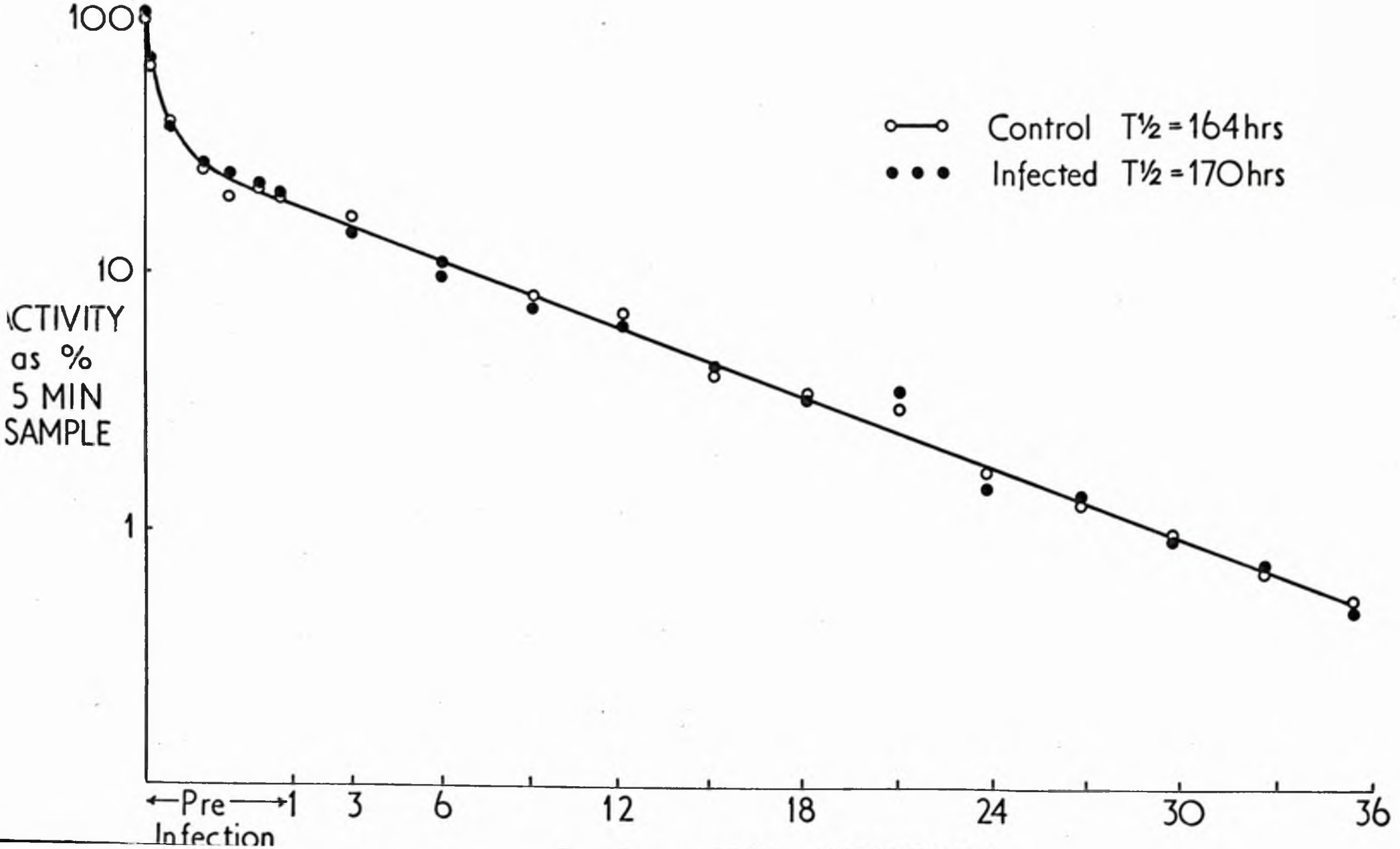
^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment in Normal and Fluke-infected Rabbits

Phase 1

Packed Cell Volumes and Serum Protein Data

	Rabbit No.	P.C.V	Total Protein (g%)	Albumin (g%)	Globulin (g%)	A/G	Globulin (g%)		
							Alpha	Beta	Gamma
CONTROL	342	40	6.25	3.23	3.02	1.07	0.54	1.47	1.03
	343	37	6.25	2.93	3.32	0.88	0.84	1.74	0.76
	344	40	6.60	3.24	3.36	0.97	0.70	1.56	1.10
	Mean	39	6.37	3.13	3.23	0.97	0.69	1.59	0.96
	S.D.	1.70	0.20	0.19	0.19	0.09	0.15	0.14	0.18
INFECTED	280	42	6.10	3.30	2.80	1.17	0.60	1.57	0.64
	303	39	5.85	3.20	2.65	1.25	0.57	1.45	0.63
	306	40	6.60	3.26	3.34	0.98	0.60	1.79	0.97
	Mean	40	6.18	3.25	2.93	1.13	0.59	1.60	0.75
	S.D.	1.53	0.38	0.05	0.36	0.14	0.02	0.17	0.19
	P	>0.10	>0.10	>0.10	>0.10	>0.10	>0.10	>0.10	>0.10

^{125}I-LABELLED ALBUMIN DISAPPEARANCE CURVE FOLLOWING INFECTION with F. HEPATICA PHASE 1 DAY 1-38 POST-INFECTION



^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiments in Rabbits Following Infection with *F. hepatica*

Phase 1 - Day 0 - 38 Post-Infection

^{125}I Labelled Albumin - Experimental Results

	Infected			Control		
	306	280	303	342	343	344
Plasma Volume (ml/Kg)	40.4	32.7	36.3	36.4	40.3	36.4
CA (gm/Kg)	1.32	1.08	1.06	1.18	1.18	1.18
TA Sterling (gm/Kg)	4.26	3.59	3.79	3.81	3.47	4.07
EA Sterling (gm/Kg)	2.94	2.51	2.73	2.63	2.29	2.89
TA (Campbell, gm/Kg)	3.73	3.20	3.51	3.41	3.32	3.50
EA (Campbell, gm/Kg)	2.41	2.12	2.45	2.23	2.14	2.32
EA/CA (Sterling)	2.27	2.33	2.58	2.23	1.94	2.45
EA/CA (Campbell)	1.83	2.21	2.31	1.89	1.81	1.97
EA/TA (Sterling)	0.69	0.70	0.72	0.69	0.66	0.71
EA/TA (Campbell)	0.65	0.66	0.70	0.65	0.65	0.66
$T\frac{1}{2}$ (hours)	175	197.5	145	187	152	153
T Equil. (hours)	55	40	35	43	38	50
F (CA) (K-Campbell)	0.200	0.215	0.299	0.216	0.243	0.193
F (TA)	0.050	0.042	0.040	0.062	0.031	0.031
Plasma Clearance (ml/24 hrs)	0.46	0.50	0.56	0.47	0.66	0.61
Absolute amount albumin catabolised (gm/Kg/24 hr)	0.264	0.235	0.317	0.255	0.287	0.228

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiments in Normal and Fluke-infected Rabbits
Phase 1: Day 0 - 38 Post-infection
Daily Plasma Clearances

bit No. 342 - Control

Day of Expt.	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Plasma Clearance (ml)
0	4,922			0.12
1	9,110	14,032	0.40	0.62
2	5,508	19,540	0.56	0.49
3	5,529	25,069	0.72	0.58
4	3,876	28,945	0.83	0.44
5	3,082	32,027	0.92	0.39
6	2,791	34,818	1.00	0.39
7	-	34,818	1.00	-
8	813	35,631	1.02	0.13
9	3,990	39,621	1.14	0.72
10	2,572	42,193	1.21	0.50
11	2,243	44,436	1.28	0.48
12	1,901	46,337	1.33	0.46
13	1,121	47,458	1.36	0.29
14	623	48,081	1.38	0.18
15	925	49,006	1.41	0.29
16	1,688	50,694	1.46	0.57
17	823	51,517	1.48	0.30
18	825	52,342	1.51	0.33
19	577	52,919	1.52	0.26
20	1,029	53,948	1.55	0.50
21	765	54,713	1.57	0.41
22	892	55,605	1.60	0.52
23	842	56,447	1.62	0.53
24	961	57,408	1.65	0.67
25	1,046	58,454	1.68	0.80
26	598	59,052	1.70	0.50
27	611	59,663	1.72	0.57
28	579	60,242	1.73	0.58
29	427	60,669	1.74	0.47
30	362	61,031	1.75	0.43
31	444	61,475	1.77	0.59
32	377	61,852	1.78	0.54
33	281	62,133	1.79	0.44
34	286	62,419	1.80	0.50
35	263	62,682	1.80	0.49
36	210	62,892	1.81	0.44
37	194	63,086	1.81	0.44
38	114	63,200	1.82	0.29
39	175	63,375	1.82	0.48
40	160	63,535	1.83	0.48
41	81	63,616	1.83	0.27
			Mean	0.47

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiments in Normal and Fluke-Infected Rabbits
Phase 1: Day 0 - 38 Post-Infection
Daily Plasma Clearances

Rabbit No. 343 - Control

Day of Expt.	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Ini.	Plasma Clearance (ml)
0	3,489			0.07
1	9,759	13,248	0.39	0.54
2	3,884	17,132	0.51	0.28
3	8,200	25,332	0.75	0.70
4	8,147	33,479	0.99	0.78
5	6,346	39,825	1.18	0.68
6	7,409	47,234	1.40	0.89
7	7,642	54,876	1.62	1.00
8	1,393	56,269	1.67	0.21
9	2,816	59,085	1.75	0.47
10	5,473	64,558	1.92	1.04
11	3,095	67,653	2.01	0.66
12	1,097	68,750	2.04	0.26
13	1,655	70,405	2.09	0.44
14	2,245	72,651	2.16	0.66
15	2,039	74,690	2.22	0.68
16	1,773	76,463	2.27	0.66
17	1,441	77,904	2.31	0.60
18	616	78,520	2.33	0.29
19	846	80,356	2.35	0.44
20	990	80,356	2.38	0.58
21	543	80,899	2.40	0.36
22	838	81,737	2.42	0.63
23	854	82,591	2.45	0.69
24	826	83,417	2.47	0.75
25	983	84,400	2.50	1.03
26	575	84,975	2.52	0.67
27	739	85,714	2.54	0.97
28	485	86,199	2.56	0.70
29	379	86,578	2.57	0.61
30	294	86,872	2.58	0.54
31	407	87,279	2.59	0.83
32	407	87,686	2.60	0.93
33	365	88,051	2.61	0.92
34	348	88,399	2.62	0.99
35	207	88,606	2.63	0.67
36	161	88,767	2.63	0.58
37	155	88,922	2.63	0.63
38	138	89,060	2.64	0.62
39	160	89,220	2.65	0.82
40	108	89,328	2.65	0.61
41	58	89,386	2.65	0.38
			Mean	0.66

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiments in Normaland Fluke Infected Rabbits
Phase 1 - Day 0 - 38 Post-Infection
Daily Plasma Clearances

Rabbit No. 344 - Control

Day of Expt.	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Plasma Clearance (ml)
0	5,327			0.10
1	9,689	15,016	0.40	0.43
2	8,332	23,348	0.62	0.53
3	6,365	29,713	0.79	0.56
4	7,328	37,041	0.98	0.76
5	4,190	41,231	1.09	0.48
6	4,394	45,625	1.21	0.56
7	6,462	52,087	1.38	0.94
8	5,752	57,839	1.53	0.92
9	2,846	60,685	1.60	0.52
10	3,281	63,966	1.69	0.67
11	2,295	66,261	1.75	0.52
12	1,223	67,484	1.78	0.31
13	2,771	70,255	1.86	0.80
14	1,059	71,314	1.89	0.34
15	1,238	72,552	1.92	0.44
16	1,085	73,637	1.95	0.43
17	1,521	75,158	1.99	0.68
18	1,069	76,227	2.02	0.54
19	485	76,712	2.03	0.27
20	741	77,453	2.05	0.46
21	405	77,858	2.06	0.28
22	953	78,811	2.08	0.73
23	741	79,552	2.10	0.65
24	576	80,128	2.12	0.55
25	820	80,948	2.14	0.90
26	331	81,279	2.15	0.40
27	304	81,583	2.16	0.42
28	243	81,826	2.16	0.36
29	305	82,131	2.17	0.51
30	338	82,469	2.18	0.65
31	214	82,683	2.19	0.46
32	235	82,918	2.19	0.57
33	191	83,109	2.20	0.51
34	313	83,422	2.21	0.94
35	212	83,634	2.21	0.70
36	218	83,852	2.22	0.82
37	168	84,020	2.22	0.70
38	117	84,137	2.22	0.56
39	271	84,408	2.23	1.45
40	123	84,531	2.23	0.74
41	135	84,666	2.24	0.89
			Mean	0.61

---I Labelled Albumin / ---Cr Labelled Erythrocytes Experiments

In Normal and Fluke-Infected Rabbits

Phase 1 - Day 0 - 38 Post-Infection

Daily Plasma Clearances

Rabbit No. 280 - Infected

Day of Expt.	Days Post Mortem	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Ini.	Plasma Clearance (ml)
0		8,491			0.17
1		15,167	23,658	0.66	0.82
2		9,828	33,486	0.93	0.69
3		6,206	39,692	1.10	0.51
4	1	5,591	45,283	1.26	0.50
5	2	7620	52,903	1.47	0.75
6	3	5,827	58,730	1.63	0.63
7	4	6,503	65,233	1.81	0.77
8	5	4,635	69,868	1.94	0.59
9	6	3,300	73,168	2.03	0.46
10	7	2,917	76,085	2.11	0.45
11	8	2,999	79,084	2.20	0.51
12	9	2,483	81,567	2.27	0.47
13	10	2,276	83,843	2.33	0.47
14	11	1,274	85,117	2.37	0.28
15	12	2,165	87,282	2.43	0.54
16	13	1,592	88,874	2.47	0.44
17	14	1,607	90,481	2.52	0.49
18	15	1,119	91,600	2.55	0.37
19	16	1,000	92,600	2.57	0.38
20	17	1,274	93,874	2.61	0.51
21	18	1,033	94,907	2.64	0.46
22	19	1,307	96,214	2.68	0.64
23	20	1,310	97,524	2.71	0.69
24	21	675	98,199	2.73	0.40
25	22	683	98,882	2.75	0.45
26	23	321	99,203	2.76	0.22
27	24	305	99,508	2.77	0.24
28	25	540	100,048	2.78	0.46
29	26	519	100,567	2.80	0.48
30	27	618	101,185	2.81	0.64
31	28	588	101,773	2.83	0.68
32	29	483	102,256	2.84	0.59
33	30	489	102,745	2.86	0.64
34	31	476	103,221	2.87	0.72
35	32	341	103,562	2.88	0.56
36	33	315	103,877	2.89	0.56
37	34	247	104,124	2.90	0.49
38	35	26	104,150	2.91	0.06
39	36	52	104,202	2.90	0.12
40	37	197	104,399	2.90	0.52
41	38	139	104,538	2.91	0.41
				Mean	0.50

In Normal and Fluke-Infected Rabbits

Phase 1 - Day 0 - 38 Post Infection

Daily Plasma Clearances

Rabbit No. 303 - Infected

Day of Expt.	Days Post Infection	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Plasma Clearance (ml)
0		6,327			0.14
1		8,176	14,503	0.44	0.55
2		4,945	19,448	0.60	0.45
3		3,736	23,184	0.71	0.39
4	1	4,514	27,698	0.85	0.54
5	2	4,237	31,935	0.98	0.58
6	3	3,390	35,325	1.08	0.53
7	4	3,206	38,531	1.18	0.57
8	5	5,383	43,914	1.35	0.18
9	6	5,959	49,873	1.53	1.38
10	7	3,214	53,097	1.63	0.84
11	8	1,398	54,485	1.67	0.42
12	9	578	55,063	1.69	0.20
13	10	497	55,560	1.70	0.20
14	11	1,628	57,188	1.75	0.73
15	12	1,479	58,667	1.80	0.76
16	13	209	58,876	1.81	0.12
17	14	382	59,258	1.82	0.26
18	15	490	59,748	1.83	0.37
19	16	502	60,250	1.85	0.43
20	17	-	60,250	1.85	-
21	18	365	60,615	1.86	0.41
22	19	351	60,966	1.87	0.46
23	20	452	61,418	1.88	0.66
24	21	282	61,700	1.89	0.48
25	22	350	62,050	1.90	0.67
26	23	220	62,270	1.91	0.49
27	24	177	62,447	1.92	0.44
28	25	84	62,531	1.92	0.24
29	26	118	62,649	1.92	0.40
30	27	110	62,759	1.93	0.42
31	28	261	63,020	1.93	1.11
32	29	202	63,222	1.94	0.99
33	30	60	63,282	1.94	0.34
34	31	142	63,424	1.95	0.89
35	32	90	63,514	1.95	0.66
36	33	63	63,577	1.95	0.51
37	34	63	63,640	1.95	0.61
38	35	25	63,665	1.95	0.28
39	36	68	63,733	1.96	0.86
40	37	31	63,764	1.96	0.46
41	38	54	63,818	1.96	0.89

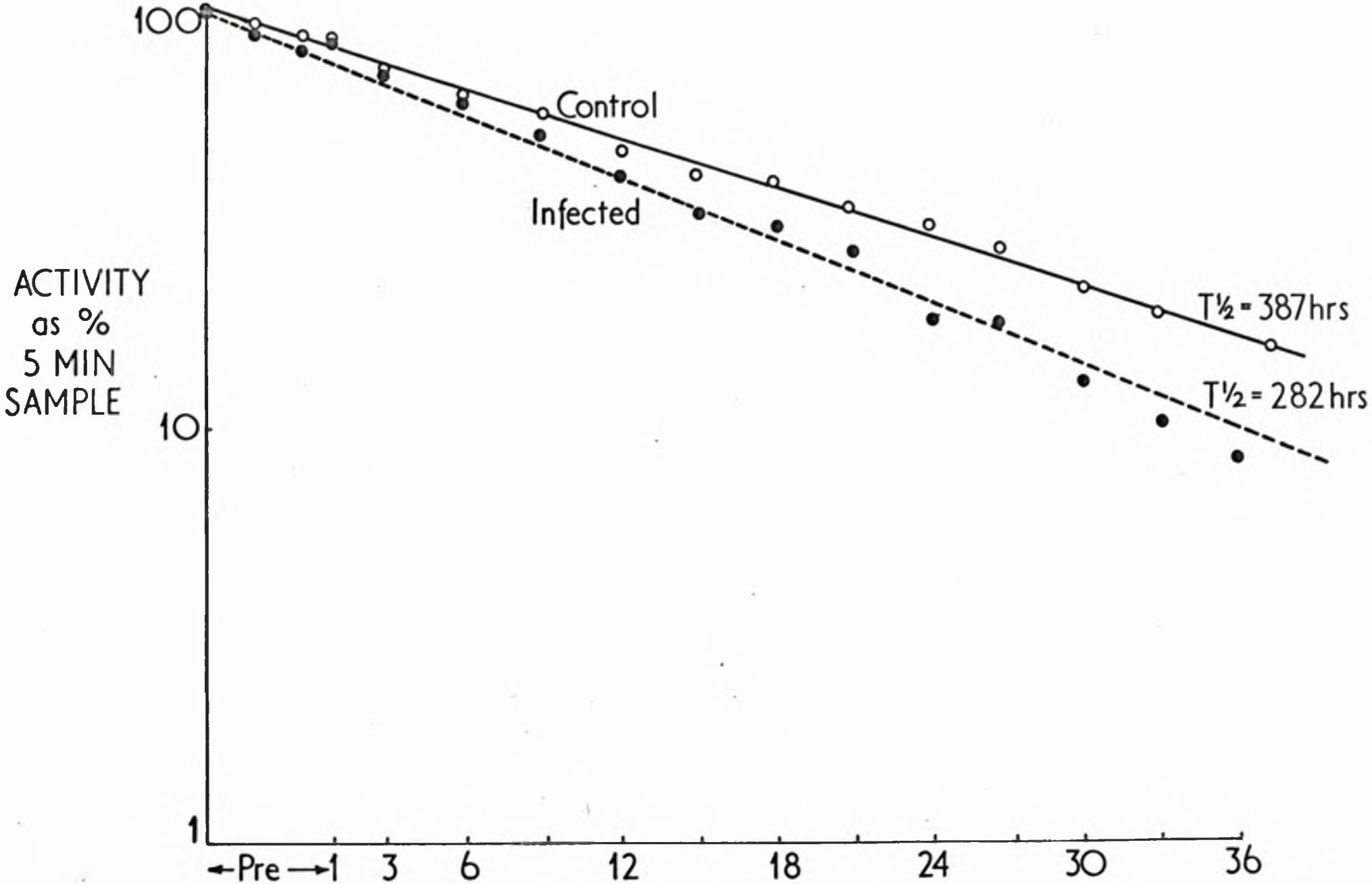
Mean 0.56

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiments in Normal and Fluke-Infected Rabbits
Phase 1 - Day 0 - 38 Post-Infection
Daily Plasma Clearances

Rabbit No. 306 - Infected

Day of Expt.	Days Post Infection	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Plasma Clearance (ml)
0		3,668			0.07
1		8,525	12,193	0.32	0.40
2		8,177	20,370	0.54	0.50
3		7,790	28,160	0.74	0.59
4	1	8,638	36,798	0.97	0.80
5	2	4,938	41,736	1.10	0.51
6	3	-	41,736	1.10	-
7	4	7,422	49,158	1.30	0.94
8	5	7,698	56,856	1.50	1.08
9	6	5,661	62,517	1.65	0.86
10	7	2,501	65,018	1.72	0.42
11	8	1,428	66,446	1.76	0.26
12	9	2,175	68,621	1.81	0.46
13	10	1,887	70,508	1.86	0.44
14	11	1,509	72,017	1.90	0.37
15	12	1,408	73,425	1.94	0.39
16	13	1,201	74,626	1.97	0.36
17	14	1,061	75,687	2.00	0.35
18	15	1,077	76,764	2.03	0.40
19	16	819	77,583	2.05	0.33
20	17	226	77,809	2.06	0.10
21	18	602	78,411	2.07	0.30
22	19	397	78,808	2.08	0.22
23	20	190	78,998	2.09	0.11
24	21	582	79,580	2.10	0.38
25	22	405	79,985	2.12	0.29
26	23	383	80,368	2.13	0.30
27	24	383	80,751	2.14	0.34
28	25	262	81,013	2.14	0.25
29	26	171	81,184	2.15	0.18
30	27	302	81,486	2.16	0.36
31	28	803	82,289	2.18	1.02
32	29	584	82,873	2.19	0.83
33	30	429	83,302	2.20	0.67
34	31	326	83,628	2.21	0.55
35	32	236	83,864	2.22	0.45
36	33	75	83,939	2.22	0.16
37	34	327	84,176	2.23	0.55
38	35	214	84,390	2.23	0.64
39	36	226	84,616	2.24	0.31
40	37	100	84,716	2.24	0.39
41	38	118	84,834	2.24	0.40
					Mean 0.46

^{51}Cr-LABELLED ERYTHROCYTES R.B.C. ACTIVITY FOLLOWING INFECTION with F. HEPATICA PHASE 1 DAY 1 -38 POST-INFECTION



^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiments in Rabbits Following Infection with F. hepatica

Phase 1: Day 0 - 38 Post-Infection

^{51}Cr Labelled Erythrocytes - Experimental Results

	Rabbit No.	Blood Volume (ml)	Blood Volume (ml/Kg)	Circulating Red Cell Volume (ml)	Circulating Red Cell Volume (ml/Kg)	Whole Blood T½ (hrs)	Red Cell T½ (hrs)	Faecal "Clearance" Whole Blood (ml/24hrs)	Faecal "Clearance" R.B.C. (ml/24 hrs)
CONTROL	342	137.5	57.3	50.2	20.9	358	358	0.11	0.04
	343	112.2	64.1	41.6	23.8	474	474	0.10	0.04
	344	116.3	58.2	43.6	21.8	328	330	0.26	0.10
INFECTED	280	119.7	55.7	49.3	22.9	258	261	0.13	0.05
	303	104.5	47.5	41.9	16.8	219	246	0.20	0.06
	306	116.6	61.4	39.9	21.0	345	340	0.14	0.05

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiments in Normal and Fluke-Infected Rabbits
Daily Whole Blood and Red Cell Clearances

Rabbit No. 342 - Control

Day of Expt.	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Whole Blood Clearance (ml)	R.B.C. Clearance (ml)
0	60				
1	114	174	0.13	0.09	0.03
2	87	261	0.20	0.07	0.03
3	128	389	0.30	0.10	0.04
4	203	592	0.45	0.18	0.07
5	102	694	0.53	0.09	0.04
6	77	771	0.59	0.08	0.03
7	114	885	0.67	0.10	0.04
8	34	919	0.70	0.04	0.01
9	133	1,052	0.80	0.10	0.05
10	101	1,153	0.88	0.10	0.04
11	105	1,258	0.95	0.10	0.05
12	78	1,336	1.01	0.10	0.04
13	53	1,389	1.05	0.07	0.03
14	48	1,437	1.08	0.07	0.03
15	54	1,491	1.13	0.08	0.03
16	68	1,559	1.18	0.10	0.04
17	61	1,620	1.23	0.10	0.04
18	63	1,683	1.28	0.10	0.04
19	33	1,716	1.30	0.06	0.02
20	47	1,763	1.34	0.09	0.03
21	40	1,803	1.37	0.08	0.03
22	47	1,850	1.40	0.09	0.04
23	60	1,910	1.45	0.13	0.05
24	67	1,977	1.50	0.15	0.06
25	75	2,052	1.56	0.17	0.07
26	41	2,093	1.59	0.10	0.04
27	41	2,134	1.62	0.10	0.04
28	47	2,181	1.65	0.13	0.05
29	54	2,235	1.70	0.16	0.06
30	37	2,272	1.72	0.11	0.04
31	52	2,324	1.76	0.16	0.06
32	56	2,380	1.81	0.18	0.07
33	21	2,401	1.82	0.07	0.03
34	30	2,431=	1.84	0.10	0.04
35	53	2,484	1.88	0.20	0.08
36	31	2,515	1.91	0.12	0.05
37	50	2,565	1.95	0.21	0.08
38	14	2,579	1.96	0.06	0.02
39	35	2,614	1.98	0.16	0.06
40	41	2,655	2.01	0.19	0.07
41	18	2,673	2.03	0.09	0.03
				Mean 0.11	Mean 0.04

[illegible] Labelled Albumin / [illegible] Excretion Experiments
in Normal and Fluke-Infected Rabbits
Daily Whole Blood and Red Cell Clearances

Rabbit No. 343 - Control

Day of Expt.	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Whole Blood Clearance (ml)	R.B.C. Clearance (ml)
0	47				
1	145	192	0.12	0.08	0.03
2	498	690	0.44	0.27	0.10
3	116	806	0.51	0.07	0.02
4	109	915	0.58	0.06	0.02
5	250	1,165	0 74	0.15	0.06
6	250	1,415	0.90	0.15	0.06
7	362	1,777	1.13	0.23	0.09
8	36	1,813	1.15	0.02	0.009
9	102	1,915	1.22	0.07	0.03
10	128	2,043	1.30	0.09	0.04
11	328	2,371	1.51	0.25	0.09
12	140	2,511	1.59	0.11	0.04
13	277	2,788	1.77	0.22	0.08
14	-	2,788	1.77	-	-
15	352	3,140	1.99	0.30	0.11
16	237	3,377	2.14	0.21	0.08
18	155	3,532	2.24	0.14	0.05
18	101	3,633	2.31	0.10	0.04
19	68	3,701	2.35	0.07	0.03
20	73	3,774	2.39	0.08	0.03
21	46	3,820	2.43	0.05	0.02
22	69	3,889	2.47	0.08	0.03
23	71	3,960	2.51	0.08	0.03
24	53	4,013	2.55	0.06	0.02
25	60	4,073	2.59	0.07	0.03
26	35	4,108	2.61	0.05	0.02
27	90	4,198	2.67	0.12	0.05
28	65	4,263	2.71	0.09	0.03
29	37	4,300	2.73	0.05	0.02
30	33	4,333	2.75	0.05	0.02
31	44	4,377	2.78	0.07	0.03
32	37	4,414	2.80	0.06	0.02
33	48	4,462	2.83	0.08	0.03
34	59	4,521	2.87	0.10	0.04
35	32	4,553	2.89	0.06	0.02
36	29	4,582	2.91	0.05	0.02
37	35	4,617	2.93	0.07	0.03
38	33	4,650	2.95	0.07	0.02
39	43	4,693	2.98	0.09	0.03
40	38	4,731	3.00	0.08	0.03
41	25	4,756	3.02	0.05	0.02
			Mean	0.10	Mean 0.04

[illegible] Labelled Albumin [illegible]

in Normal and Fluke-Infected Rabbits

Daily Whole Blood and Red Cell Clearances

Rabbit No. 344 - Control

Day of Expt.	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Whole Blood Clearance (ml)	R.B.C. Clearance (ml)
0	75				
1	131	206	0.20	0.21	0.08
2	121	327	0.32	0.20	0.08
3	116	443	0.43	0.20	0.08
4	115	558	0.54	0.21	0.08
5	91	649	0.63	0.18	0.07
6	83	732	0.71	0.17	0.07
7	143	875	0.85	0.31	0.11
8	161	1,036	1.00	0.37	0.14
9	83	1,119	1.08	0.20	0.08
10	67	1,186	1.15	0.17	0.06
11	45	1,231	1.19	0.12	0.05
12	30	1,261	1.22	0.08	0.03
13	91	1,352	1.31	0.26	0.10
14	52	1,404	1.36	0.16	0.06
15	36	1,440	1.39	0.12	0.05
16	99	1,539	1.49	0.33	0.13
17	98	1,637	1.58	0.35	0.14
18	97	1,734	1.68	0.36	0.14
19	59	1,793	1.73	0.23	0.09
20	41	1,834	1.77	0.17	0.06
21	24	1,858	1.80	0.10	0.04
22	-	1,858	1.80	-	-
23	54	1,912	1.85	0.26	0.10
24	45	1,957	1.89	0.23	0.08
25	86	2,043	1.97	0.46	0.18
26	36	2,079	2.00	0.20	0.08
27	17	2,096	2.03	0.10	0.04
28	25	2,121	2.05	0.16	0.06
29	37	2,158	2.09	0.24	0.09
30	30	2,188	2.11	0.21	0.08
31	33	2,221	2.15	0.24	0.09
32	32	2,253	2.18	0.24	0.09
33	26	2,279	2.20	0.21	0.08
34	49	2,328	2.25	0.41	0.16
35	50	2,378	2.30	0.44	0.17
36	36	2,414	2.33	0.33	0.13
37	41	2,455	2.37	0.39	0.16
38	37	2,492	2.41	0.38	0.15
39	67	2,559	2.47	0.72	0.28
40	32	2,591	2.50	0.36	0.14
41	32	2,623	2.53	0.39	0.15
				Mean 0.26	Mean 0.13

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiments in Normal and Fluke-Infected Rabbits
Daily Whole Blood and Red Cell Clearances

Rabbit No. 280 - Infected

Day of Expt.	Days Post Infection	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Whole Blood Clearance (ml)	R.B.C. Clearance (ml)
0		76				
1		205	281	0.17	0.11	0.05
2		192	473	0.29	0.11	0.05
3		95	568	0.34	0.06	0.02
4	1	102	670	0.40	0.06	0.03
5	2	114	784	0.47	0.08	0.03
6	3	112	896	0.54	0.08	0.03
7	4	121	1,017	0.61	0.09	0.04
8	5	108	1,125	0.68	0.09	0.04
9	6	93	1,218	0.74	0.08	0.03
10	7	66	1,284	0.78	0.06	0.033
11	8	73	1,357	0.82	0.07	0.03
12	9	77	1,434	0.87	0.08	0.03
13	10	110	1,544	0.93	0.12	0.05
14	11	83	1,627	0.98	0.10	0.04
15	12	93	1,720	1.04	0.12	0.05
16	13	75	1,795	1.08	0.10	0.04
17	14	93	1,888	1.14	0.13	0.06
18	15	51	1,939	1.17	0.08	0.03
19	16	46	1,985	1.20	0.08	0.03
20	17	83	2,068	1.25	0.14	0.06
21	18	55	2,123	1.28	0.10	0.04
22	19	66	2,189	1.32	0.13	0.05
23	20	79	2,268	1.37	0.17	0.07
24	21	19	2,287	1.38	0.04	0.02
25	22	39	2,326	1.40	0.10	0.04
26	23	72	2,398	1.45	0.19	0.08
27	24	60	2,458	1.48	0.17	0.07
28	25	44	2,502	1.51	0.13	0.05
29	26	31	2,533	1.53	0.10	0.04
30	27	56	2,589	1.56	0.19	0.08
31	28	64	2,653	1.60	0.23	0.09
32	29	53	2,706	1.63	0.21	0.08
33	30	52	2,758	1.66	0.21	0.08
34	31	35	2,793	1.69	0.15	0.06
35	32	63	2,856	1.72	0.29	0.12
36	33	48	2,904	1.75	0.24	0.09
37	34	35	2,939	1.77	0.18	0.07
38	35	27	2,966	1.79	0.15	0.06
39	36	43	3,009	1.82	0.26	0.10
40	37	41	3,050	1.84	0.26	0.10
41	38	23	3,073	1.86	0.16	0.06
					Mean 0.13	Mean 0.05

I Labelled Albumin / ... Of Labelled Erythrocytes Intravenously

In Normal and Fluke-Infected Rabbits

Daily Whole Blood and Red Cell Clearances

Rabbit No. 303 - Infected

ay of Expt.	Days Post Infection	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Whole Blood Clearance (ml)	R.B.C. Clearance (ml)
0		119				
1		145	264	0.15	0.07	0.03
2		85	349	0.20	0.04	0.02
3		66	415	0.24	0.04	0.01
4	1	105	520	0.29	0.06	0.02
5	2	99	619	0.35	0.06	0.02
6	3	70	689	0.39	0.05	0.02
7	4	86	775	0.44	0.06	0.02
8	5	161	936	0.53	0.11	0.04
9	6		936	0.53	-	-
10	7	128	1,064	0.60	0.11	0.04
11	8	69	1,133	0.64	0.06	0.02
12	9	79	1,212	0.69	0.07	0.03
13	10	63	1,275	0.72	0.06	0.02
14	11	83	1,358	0.77	0.09	0.03
15	12	88	1,446	0.82	0.10	0.04
16	13	52	1,498	0.85	0.06	0.02
17	14	94	1,592	0.90	0.12	0.05
18	15	64	1,656	0.94	0.09	0.03
19	16	36	1,692	0.96	0.05	0.02
20	17	86	1,778	1.00	0.13	0.05
21	18	105	1,883	1.07	0.17	0.07
22	19	60	1,943	1.10	0.10	0.04
23	20	100	2,043	1.16	0.18	0.08
24	21	108	2,151	1.22	0.19	0.09
25	22	74	2,225	1.26	0.16	0.06
26	23	53	2,278	1.29	0.12	0.05
27	24	72	2,350	1.33	0.18	0.07
28	25	64	2,414	1.37	0.17	0.07
29	26	41	2,455	1.39	0.13	0.05
30	27	45	2,500	1.42	0.15	0.06
31	28	169	2,669	1.51	0.65	0.22
32	29	110	2,779	1.58	0.46	0.14
33	30	77	2,856	1.62	0.35	0.12
34	31	103	2,989	1.68	0.53	0.17
35	32	50	3,009	1.71	0.29	0.09
36	33	60	3,069	1.74	0.39	0.11
37	34	60	3,129	1.77	0.41	0.12
38	35	67	3,196	1.81	0.52	0.14
39	36	75	3,271	1.85	0.64	0.17
40	37	47	3,318	1.88	0.44	0.11
41	38	41	3,359	1.90	0.43	0.10
					Mean 0.20	Mean 0.06

I Labelled Albumin / Of Normal Erythrocytes Suspensions

in Normal and Fluke-Infected Rabbits

Daily Whole Blood and Red Cell Clearances

bbit No. 306 - Infected

ay of Expt.	Days Post Infection	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Whole Blood Clearance (ml)	R.B.C. Clearance (ml)
0		164				
1		353	517	0.23	0.27	0.10
2		156	673	0.30	0.12	0.05
3		128	801	0.36	0.10	0.04
4	1	222	1,023	0.46	0.20	0.07
5	2	-	1,023	0.46	-	-
6	3	164	1,187	0.53	0.15	0.06
7	4	251	1,438	0.64	0.25	0.09
8	5	339	1,777	0.80	0.35	0.10
9	6	198	1,975	0.89	0.22	0.08
10	7	73	2,048	0.92	0.08	0.03
11	8	29	2,077	0.93	0.04	0.01
12	9	91	2,168	0.97	0.10	0.04
13	10	91	2,259	1.01	0.12	0.05
14	11	81	2,340	1.05	0.10	0.04
15	12	90	2,430	1.09	0.13	0.05
16	13	102	2,532	1.14	0.16	0.06
17	14	86	2,618	1.17	0.13	0.05
18	15	84	2,702	1.21	0.14	0.06
19	16	79	2,781	1.25	0.14	0.05
20	17	52	2,833	1.27	0.10	0.04
21	18	52	2,885	1.29	0.10	0.04
22	19	44	2,929	1.31	0.09	0.04
23	20	21	2,950	1.32	0.05	0.02
24	21	38	2,988	1.34	0.09	0.03
25	22	25	3,013	1.35	0.06	0.02
26	23	-	3,013	1.35	-	-
27	24	46	3,059	1.37	0.12	0.05
28	25	25	3,084	1.38	0.07	0.03
29	26	16	3,100	1.39	0.05	0.02
30	27	61	3,161	1.42	0.19	0.07
31	28	82	3,243	1.45	0.27	0.10
32	29	71	3,314	1.49	0.24	0.09
33	30	61	3,375	1.51	0.22	0.08
34	31	62	3,437	1.54	0.23	0.09
35	32	27	3,464	1.55	0.10	0.04
36	33	18	3,482	1.56	0.07	0.03
37	34	21	3,503	1.57	0.09	0.04
38	35	19	3,522	1.58	0.09	0.03
39	36	20	3,542	1.59	0.10	0.04
40	37	14	3,556	1.59	0.07	0.03
41	38	20	3,576	1.60	0.10	0.04
					Mean 0.14	Mean 0.05

^{125}I-LABELLED ALBUMIN/ ^{51}Cr-LABELLED ERYTHROCYTES EXPERIMENTS

PHASE 2:-EXPERIMENTAL RESULTS

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment in Normal and Fluke Infected Rabbits

Phase 2.

Packed Cell Volumes and Serum Protein Data

	Rabbit No.	P.C.V.	Total Protein (g/%)	Albumin (g%)	Globulin (g%)	A/G	Globulin /g%)		
							Alpha	Beta	Gamma
CONTROL	199	39	6.60	3.25	3.35	0.97	0.53	1.39	1.44
	312	40	6.10	3.33	2.77	1.20	0.63	1.31	0.88
	317	38	6.10	3.05	3.05	1.00	0.42	1.80	0.83
	Mean	39	6.27	3.21	3.06	1.06	0.53	1.50	1.05
	S.D.	1.00	0.29	0.14	0.29	0.12	0.10	0.26	0.34
INFECTED	304	37	6.60	2.47	4.13	0.60	0.95	2.16	1.02
	308	38	6.90	2.72	4.18	0.65	0.90	1.87	1.22
	310	45	7.60	2.50	5.10	0.49	0,78	3.27	1.08
	307	34	6.10	2.53	3.57	0.71	0.84	1.54	1.03
	311	41	7.00	2.81	4.19	0.67	0.72	1.82	1.65
	Mean	39	6.84	2.61	4.23	0.62	0.84	2.13	1.20
	S.D.	4.18	0.55	0.15	0.55	0.08	0.09	0.67	0.26
	P	>0.10	>0.10	<0.002	<0.02	<0.002	<0.01	>0.10	>0.10

^{125}I Labelled Albumin and ^{51}Cr Labelled Erythrocytes Experiments in Rabbits Following Infection with F. hepatica

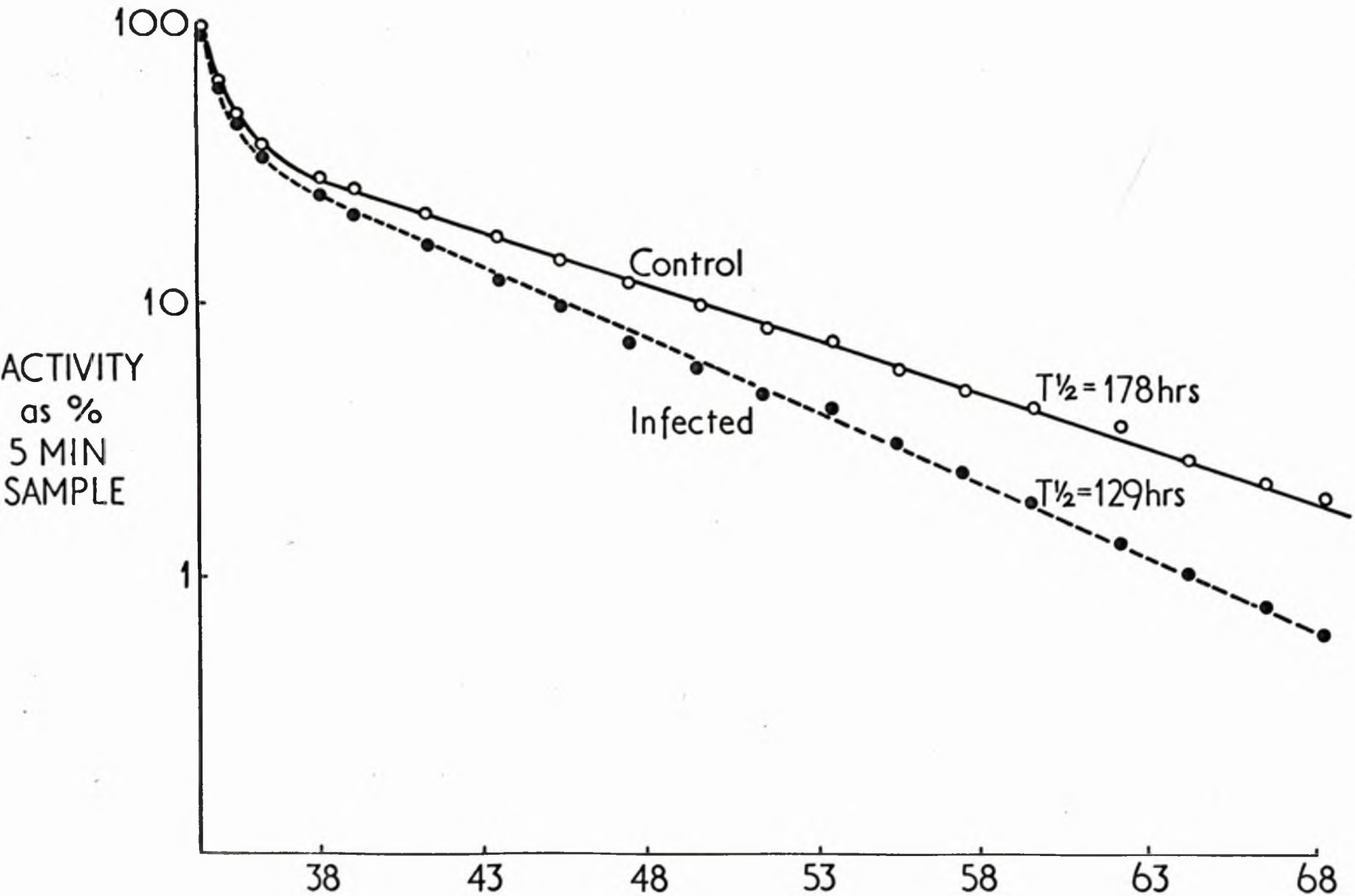
Phase 2. Day 35 - 70 Post Infection

^{51}Cr Labelled Erythrocytes - Experimental Results

Rabbit No.	Blood Volume (ml)	Blood Volume (ml/kg)	Circulating Red Cell Volume (ml)	Circulating Red Cell Volume (ml/kg)	Whole Blood T½ (hrs)	Red Cell T½ (hrs)	"Faecal Clearance" Whole Blood (ml/24hrs)	"Faecal Clearance" R.B.C. (ml/24hrs)
317	129.05	60.05	37.95	17.65	308	315	0.12	0.05
199	99.76	49.93	23.66	11.83	305	300	0.05	0.02
312	95.78	47.89	25.58	12.79	312	310	0.08	0.03
Mean	108.20	52.62	29.06	14.09	308.3	308.3	0.083	0.033
S.D.	18.17	6.51	7.76	3.12	3.51	7.64	0.035	0.014
304	100.75	45.79	27.05	12.29	173	178	2.1	0.7
307	115.79	58.46	31.69	15.46	100	117	12.2	3.4
308	112.59	57.53	30.29	15.53	205	210	2.9	1.0
310	97.69	55.80	28.87	16.50	135	140	7.6	2.1
311	96.65	52.29	25.15	13.59	160	170	6.1	1.6
Mean	104.69	53.41	28.61	14.67	154.6	163.0	6.18	1.76
S.D.	8.87	4.69	2.59	1.70	39.6	35.81	4.05	1.06
P	> 0.10	> 0.10	> 0.10	> 0.10	< 0.01	< 0.001	< 0.001	< 0.05

Note: Infected Faecal Clearances Calculated From Day 56 - 70 Post-Infection.

^{125}I-LABELLED ALBUMIN DISAPPEARANCE CURVES FOLLOWING INFECTION with F HEPATICA. PHASE 2 DAY 35-70 POST-INFECTION.



^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiments in Rabbits Following Infection with F. hepatica
Phase 2. Day 35 - 70 Post-infection

^{125}I Labelled Albumin - Experimental Results

	Infected					Control		
	304	307	308	310	311	317	199	312
Plasma Volume (ml/Kg)	33.5	41.0	42.2	39.3	38.7	42.4	38.1	35.1
Ca (gm/Kg)	0.827	1.04	1.148	0.98	1.09	1.293	1.238	1.17
TA (Sterling, gm/Kg)	2.584	3.25	2.94	2.58	3.30	2.751	3.346	3.079
EA (Sterling, gm/Kg)	1.757	2.21	1.79	1.60	2.21	1.458	2.108	1.909
TA (Campbell, gm/Kg)	2.360	3.20	2.85	3.23	3.23	2.654	3.151	2.998
EA (Campbell, gm/Kg)	1.533	2.16	1.70	2.25	2.14	1.361	1.913	1.828
EA/CA (Sterling)	2.12	2.13	1.56	1.63	2.03	1.13	1.70	1.63
EA/CA (Campbell)	1.85	2.07	1.48	2.30	1.96	1.05	1.55	1.56
EA/TA (Sterling)	0.68	0.68	0.61	0.62	0.67	0.53	0.63	0.62
EA/TA (Campbell)	0.65	0.64	0.60	0.70	0.66	0.51	0.61	0.61
$T_{\frac{1}{2}}$ (hours)	130	118	135	127	123	175	200	160
T.equil. (hours)	50	48	50	62	62	57	52	50
F(CA) (K-Campbell)	0.300	0.352	0.270	0.290	0.310	0.180	0.170	0.215
K - Matthews	0.349	0.391	0.282	0.307	0.328	0.188	0.204	0.245
F (TA)	0.052	0.045	0.050	0.023	0.053	0.039	0.042	0.039
Plasma Clearance (ml/24 hours)	0.85	1.89	1.75	1.31	2.09	0.36	0.61	0.71
Absolute amount albumin catabolised (gm/Kg/24hours)	0.250	0.366	0.310	0.284	0.34	0.299	0.210	0.252

^{125}I-Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment in Normal and Fluke-Infected Rabbits
Phase 2. Day 35 - 70 Post Infection
Daily Plasma Clearance

Rabbit No. 317 - Control

Day of Expt.	Faecal Activity	Cumulative Activity	Cumulative Activity as % Inj.	Plasma Clearance (ml)
0	3,589		0.12	0.11
1	8,730	12,319	0.41	0.40
2	8,860	21,179	0.71	0.59
3	5,029	26,208	0.87	0.40
4	3,295	29,503	0.98	0.29
5	3,061	32,564	1.09	0.30
6	1,722	34,286	1.14	0.19
7	1,914	36,200	1.21	0.23
8	1,104	37,304	1.24	0.15
9	1,216	38,514	1.28	0.18
10	1,436	39,950	1.33	0.23
11	2,739	42,689	1.42	0.48
12	2,203	44,892	1.50	0.42
13	1,377	46,269	1.54	0.29
14	1,191	47,460	1.58	0.27
15	1,328	48,788	1.63	0.34
16	1,596	50,384	1.68	0.44
17	1,007	51,391	1.71	0.31
18	763	52,154	1.74	0.25
19	979	53,133	1.77	0.35
20	884	54,017	1.80	0.35
21	462	54,479	1.82	0.18
22	487	54,966	1.83	0.23
23	855	55,821	1.86	0.46
24	539	56,360	1.88	0.31
25	344	56,704	1.89	0.22
26	271	56,975	1.90	0.19
27	504	57,479	1.92	0.39
28	966	58,445	1.95	0.82
29	790	59,235	1.98	0.73
30	567	59,802	1.99	0.57
31	630	60,432	2.01	0.68
32	244	60,676	2.02	0.27
33	161	60,817	2.03	0.21
34	394	61,211	2.04	0.57
35	209	61,420	2.05	0.33
				Mean 0.36

^{125}I-Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment
in Normal and Fluke-Infected Rabbits
Phase 2. Day 35 - 70 Post Infection
Daily Plasma Clearance

Rabbit No. 199 - Control

Day of Expt.	Faecal Activity	Cumulative Activity	Cumulative Activity as % Ini.	Plasma Clearance (ml)
0	762			0.02
1	10,440	11,202	0.37	0.65
2	-	11,202	0.37	-
3	12,043	23,245	0.76	1.07
4	5,650	28,895	0.94	0.55
5	3,500	32,395	1.06	0.38
6	5,611	38,006	1.24	0.65
7	3,412	41,418	1.35	0.43
8	2,136	43,554	1.42	0.30
9	3,901	47,455	1.55	0.59
10	1,955	49,410	1.61	0.32
11	3,750	53,160	1.73	0.87
12	3,360	56,520	1.84	0.64
13	3,581	60,101	1.96	0.74
14	2,398	62,490	2.04	0.54
15	2,976	65,466	2.13	0.74
16	959	66,425	2.17	0.26
17	2,454	68,879	2.25	0.72
18	1,872	70,751	2.31	0.60
19	1,825	72,576	2.37	0.63
20	1,765	74,341	2.42	0.66
21	1,524	75,865	2.47	0.62
22	1,702	77,567	2.53	0.75
23	1,958	79,525	2.59	0.93
24	1,058	80,583	2.63	0.54
25	1,412	81,995	2.67	0.79
26	1,408	83,403	2.72	0.85
27	1,334	84,737	2.76	0.87
28	884	85,621	2.79	0.63
29	-	85,621	2.79	-
30	1,899	87,520	2.85	1.57
31	1,108	88,628	2.89	0.99
32	785	89,413	2.92	0.75
33	560	89,973	2.93	0.59
34	111	90,084	2.94	0.13
35	795	90,879	2.96	0.99

Mean 0.61

^{125}I-Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment in Normaland Fluke-Infected Rabbits
Phase 2. Day 35 - 70 Post Infection
Daily Plasma Clearances

Rabbit No. 312 - Control

Day of Expt.	Faecal Activity	Cumulative Activity	Cumulative Activity as % inj.	Plasma Clearance (ml)
0	1,739		0.06	0.08
1	11,725	13,454	0.45	0.79
2	6,795	20,249	0.68	0.55
3	9,546	29,795	1.00	0.89
4	5,320	35,115	1.18	0.54
5	4,946	60,061	1.34	0.56
6	5,247	45,308	1.51	0.68
7	3,814	49,122	1.64	0.56
8	2,605	51,727	1.73	0.41
9	2,895	54,622	1.83	0.50
10	2,506	57,128	1.91	0.49
11	2,846	59,974	2.00	0.62
12	2,122	62,096	2.07	0.52
13	3,769	65,865	2.20	1.03
14	2,381	68,246	2.28	0.71
15	1,931	70,177	2.35	0.63
16	1,542	71,719	2.40	0.56
17	1,126	72,845	2.44	0.47
18	1,146	73,991	2.47	0.53
19	1,435	75,426	2.52	0.73
20	1,891	77,317	2.58	1.06
21	1,579	78,896	2.64	1.00
22	899	79,795	2.67	0.64
23	706	80,501	2.69	0.55
24	1,206	81,707	2.73	1.05
25	875	82,582	2.76	0.86
26	768	83,350	2.79	0.82
27	708	84,056	2.81	0.87
28	1,247	85,305	2.85	1.67
29	582	85,887	2.87	0.88
30	1,160	87,047	2.91	0.80
31	565	87,612	2.93	1.06
32	481	88,093	2.95	0.98
33	416	88,509	2.96	0.93
34	315	88,824	2.97	0.80
35	511	89,355	2.98	1.46
				Mean 0.76

^{125}I-Labelled Albumin / ^{51}Cr-Labelled Erythrocytes Experiment in Normal and Fluke-infected Rabbits
Phase 2. Day 35 - 70 Post Infection
Daily Plasma Clearance

Rabbit No. 304 - Infected

Day of Expt.	Days Post Infection	Faecal Activity	Cumulative Faecal Activity	Cumulative as % Injected	Plasma Clearance (ml)
0	35	2,963		0.10	0.08
1	36	7,260	10,223	0.36	0.41
2	37	5,180	15,403	0.54	0.46
3	38	4,649	20,052	0.71	0.51
4	39	4,248	24,300	0.86	0.53
5	40	3,026	27,326	0.97	0.44
6	41	3,429	30,755	1.09	0.58
7	42	837	31,592	1.12	0.16
8	43	6,315	37,907	1.34	1.37
9	44	2,232	40,139	1.42	0.36
10	45	1,986	41,745	1.47	0.44
11	46	2,101	43,846	1.55	0.65
12	47	2,217	46,063	1.63	0.78
13	48	4,712	50,775	1.79	1.89
14	49	2,478	53,253	1.88	1.13
15	50	2,020	55,273	1.96	1.03
16	51	1,706	56,979	2.01	1.00
17	52	1,573	58,552	2.07	1.05
18	53	1,118	59,670	2.11	0.83
19	54	792	60,462	2.14	0.67
20	55	777	61,239	2.16	0.73
21	56	713	61,952	2.19	0.79
22	57	936	62,888	2.22	1.19
23	58	638	63,526	2.24	0.92
24	59	800	64,326	2.27	1.30
25	60	374	64,700	2.28	0.70
26	61	486	65,186	2.30	1.05
27	62	588	65,774	2.32	1.46
28	63	422	66,196	2.34	1.17
29	64	196	66,392	2.34	0.62
30	65	234	66,626	2.35	0.85
31	66	192	66,818	2.36	0.78
32	67	183	67,001	2.37	0.85
33	68	119	67,120	2.37	0.63
34	69	184	67,304	2.38	1.12
35	70	152	67,456	2.38	1.04

Mean 0.85

^{125}I-Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment in Normal and Fluke Infected Rabbits
Phase 2. Day 35 - 70 Post Infection
Daily Plasma Clearance

Rabbit No. 307 - Infected

Day of Expt.	Days Post Infection	Faecal Activity	Cumulative Activity	Cumulative Activity as % Inj.	Plasma Clearance (ml)
0	35	7,862		0.26	0.22
1	36	10,360	18,222	0.62	0.61
2	37	6,360	24,582	0.83	0.65
3	38	3,305	27,887	0.94	0.43
4	39	3,027	30,914	1.05	0.43
5	40	3,180	34,094	1.15	0.53
6	41	4,421	38,515	1.30	0.67
7	42	2,318	40,833	1.38	0.53
8	43	3,266	44,099	1.49	0.85
9	44	1,280	45,379	1.54	0.38
10	45	2,717	48,096	1.63	0.93
11	46	2,106	50,202	1.70	0.83
12	47	3,274	53,476	1.81	1.50
13	48	1,862	55,338	1.87	0.98
14	49	3,380	58,718	1.99	2.05
15	50	1,112	59,830	2.03	0.77
16	51	1,285	61,115	2.07	1.02
17	52	1,144	62,259	2.11	1.05
18	53	1,805	64,064	2.17	1.87
19	54	2,129	66,193	2.24	2.63
20	55	867	67,060	2.27	1.23
21	56	1,706	68,766	2.33	2.86
22	57	919	69,685	2.36	1.74
23	58	1,666	71,351	2.42	3.65
24	59	465	71,816	2.43	1.20
25	60	680	72,496	2.46	2.02
26	61	1,361	73,857	2.50	4.61
27	62	593	74,450	2.52	2.34
28	63	966	75,416	2.55	4.43
29	64	279	75,695	2.56	1.47
30	65	954	76,649	2.60	5.78
31	66	315	76,964	2.61	2.23
32	67	515	77,479	2.62	4.19
33	68	152	77,631	2.63	1.45
34	69	475	78,105	2.65	5.22
35	70	272	78,377	2.70	3.36

Mean 1.89

^{125}I-Labelled Albumin / ^{51}Cr-Labelled Erythrocytes Experiment in Normal and Fluke-Infected Rabbits
Phase 2. Day 35 - 70 Post Infection
Daily Plasma Clearance

Rabbit No. 308 - Infected

Day of Expt.	Days Post Infection	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Plasma Clearance (ml)
0	35	240			
1	36	7,068	7,308	0.24	0.32
2	37	11,744	19,052	0.61	0.86
3	38	9,698	28,750	0.92	0.88
4	39	6,021	34,771	1.12	0.62
5	40	12,438	47,209	1.52	1.46
6	41	2,720	49,929	1.60	0.36
7	42	620	50,549	1.62	0.10
8	43	11,428	61,977	1.99	1.89
9	44	9,745	71,722	2.30	1.84
10	45	11,253	82,975	2.67	2.38
11	46	7,163	90,138	2.90	1.72
12	47	11,752	101,890	3.27	3.17
13	48	5,886	107,776	3.46	1.81
14	49	2,866	110,642	3.55	1.00
15	50	2,564	113,206	3.64	1.00
16	51	2,870	116,076	3.73	1.24
17	52	1,388	117,464	3.77	0.68
18	53	3,635	121,099	3.89	2.04
19	54	2,495	123,594	3.97	1.57
20	55	2,079	125,673	4.04	1.49
21	56	548	126,221	4.06	0.44
22	57	3,103	129,324	4.15	2.83
23	58	1,761	131,085	4.21	1.79
24	59	1,620	132,705	4.26	1.86
25	60	1,894	134,599	4.32	2.50
26	61	1,478	136,077	4.37	2.17
27	62	1,487	137,564	4.42	2.62
28	63	2,457	140,021	4.50	4.64
29	64	1,305	141,326	4.54	2.76
30	65	420	141,746	4.55	1.01
31	66	1,063	142,809	4.59	2.93
32	67	717	143,526	4.61	2.11
33	68	764	144,290	4.64	2.65
34	69	416	144,706	4.65	1.66
35	70	600	154,306	4.67	2.64

Mean 1.75

^{125}I-Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment in Normal and Fluke Infected Rabbits
Phase 2. Day 35 - 70 Post Infection
Daily Plasma Clearance

Rabbit No. 310 - Infected

Day of Expt.	Days Post Infection	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Ini.	Plasma Clearance (ml)
0	35				
1	36				
2	37	2,523	2,523	0.09	0.22
3	38	8,653	11,176	0.41	0.90
4	39	10,964	22,140	0.81	1.14
5	40	4,823	26,963	0.98	0.58
6	41	3,592	30,555	1.11	0.50
7	42	4,549	35,104	1.28	0.71
8	43	2,626	37,740	1.38	0.47
9	44	3,709	41,449	1.51	0.74
10	45	2,174	43,623	1.59	0.49
11	46	7,358	50,981	1.86	1.92
12	47	2,926	53,907	1.96	0.87
13	48	2,460	56,367	2.05	0.83
14	49	2,638	59,005	2.15	1.00
15	50	3,377	62,382	2.28	1.54
16	51	2,978	65,360	2.38	1.46
17	52	1,268	66,628	2.43	0.71
18	53	2,434	69,262	2.52	1.52
19	54	2,330	71,592	2.60	1.67
20	55	265	71,857	2.61	0.21
21	56	2,415	74,272	2.70	2.24
22	57	1,300	75,572	2.75	1.41
23	58	1,346	76,918	2.80	1.64
24	59	760	77,678	2.83	1.06
25	60	919	78,597	2.86	1.44
26	61	811	79,408	2.89	1.45
27	62	1,250	80,658	2.93	2.61
28	63	1,663	82,321	3.00	3.96
29	64	316	82,637	3.01	0.86
30	65	480	83,117	3.02	1.47
31	66	780	83,897	3.05	2.71
32	67	651	84,548	3.08	2.63
33	68	396	84,944	3.09	1.80
34	69	200	85,144	3.10	1.04
35	70	557	85,701	3.12	3.23

Mean 1.31

^{125}I-Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment in Normal and Fluke Infected Rabbits
Phase 2. - day 35 - 70 Post Infection
Daily Plasma Clearances

Rabbit No. 311 - Infected

Day of Expt.	Days Post Infection	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Ini.	Plasma Clearance (ml)
0	35	1,728		0.06	0.04
1	36	1,920	3,656	0.13	0.09
2	37	8,321	12,777	0.44	0.61
3	38	1,402	14,179	0.49	0.13
4	39	1,761	15,940	0.55	0.19
5	40	12,005	27,945	0.97	1.49
6	41	11,130	39,075	1.36	1.54
7	42	9,431	48,506	1.69	1.47
8	43	1,295	49,801	1.73	0.23
9	44	9,141	58,942	2.05	1.75
10	45	7,491	66,433	2.31	1.62
11	46	7,042	73,475	2.56	1.67
12	47	6,200	79,675	2.77	1.64
13	48	8,122	87,797	3.06	2.41
14	49	3,967	91,764	3.20	1.32
15	50	3,341	95,105	3.31	1.24
16	51	4,530	99,635	3.47	1.89
17	52	7,881	107,536	3.74	3.83
18	53	8,938	116,474	4.05	4.54
19	54	6,422	122,896	4.28	3.63
20	55	4,742	127,638	4.44	3.03
21	56	1,672	129,310	4.50	1.20
22	57	3,949	133,259	4.64	3.07
23	58	2,104	135,363	4.71	1.87
24	59	2,556	137,919	4.80	2.54
25	60	972	138,891	4.83	1.05
26	61	2,455	141,346	4.92	3.05
27	62	2,710	144,056	5.01	3.75
28	63	2,604	146,660	5.10	4.05
29	64	1,862	148,522	5.17	3.19
30	65	3,640	152,162	5.30	6.97
31	66	876	153,038	5.33	1.85
32	67	1,284	154,322	5.37	3.04
33	68	646	154,968	5.39	1.71
34	69	584	155,552	5.41	1.73
35	80	660	156,212	5.44	2.16

Mean 2.09

^{125}I Labelled Albumin and ^{51}Cr Labelled Erythrocytes Experiments in Rabbits Following Infection with F. hepatica

Phase 2. Day 35 - 70 Post Infection

^{51}Cr Labelled Erythrocytes - Experimental Results

Rabbit No.	Blood Volume (ml)	Blood Volume (ml/kg)	Circulating Red Cell Volume (ml)	Circulating Red Cell Volume (ml/kg)	Whole Blood $T\frac{1}{2}$ (hrs)	Red Cell $T\frac{1}{2}$ (hrs)	"Faecal Clearance" Whole Blood (ml/24hrs)	"Faecal Clearance" R.B.C. (ml/24hrs)
317	129.05	60.05	37.95	17.65	308	315	0.12	0.05
199	99.76	49.93	23.66	11.83	305	300	0.05	0.02
312	93.78	47.89	25.58	12.79	312	310	0.08	0.03
Mean	108.20	52.62	29.06	14.09	308.3	308.3	0.083	0.033
S.D.	18.17	6.51	7.76	3.12	3.51	7.64	0.035	0.014
304	100.73	45.79	27.05	12.29	173	178	2.1	0.7
307	115.79	58.46	31.69	15.46	100	117	12.2	3.4
308	112.59	57.53	30.29	15.53	205	210	2.9	1.0
310	97.69	55.80	28.87	16.50	135	140	7.6	2.1
311	96.65	52.29	25.15	13.59	160	170	6.1	1.6
Mean	104.69	53.41	28.61	14.67	154.6	163.0	6.18	1.76
S.D.	8.87	4.69	2.59	1.70	39.6	35.81	4.05	1.06
P	> 0.10	> 0.10	> 0.10	> 0.10	< 0.01	< 0.001	< 0.001	< 0.05

Note. Infected Faecal Clearances Calculated From Day 56 - 70 Post-Infection.

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment in Normal and Fluke Infected Rabbits
Phase 2. Day 35 - 70 Post Infection
Daily Whole Blood and Red Cell Clearances

Rabbit No. 317 - Control

Day of Expt.	Faecal Activity	Cumulative Activity	Cumulative Activity as % Inj.	Whole Blood Clearance (ml)	R.B.C. Clearance (ml)
0	46				
1	245	291	0.07	0.06	0.03
2	615	906	0.22	0.17	0.07
3	418	1,324	0.32	0.12	0.05
4	315	1,639	0.40	0.10	0.04
5	302	1,941	0.48	0.10	0.04
6	181	2,122	0.52	0.06	0.03
7	228	2,350	0.58	0.08	0.03
8	198	2,548	0.62	0.07	0.03
9	133	2,681	0.66	0.05	0.02
10	184	2,865	0.70	0.08	0.03
11	337	3,202	0.78	0.15	0.06
12	245	3,447	0.85	0.11	0.05
13	229	3,676	0.90	0.11	0.05
14	227	3,903	0.96	0.12	0.05
15	135	4,038	1.00	0.10	0.04
16	418	4,506	1.10	0.24	0.10
17	256	4,762	1.17	0.16	0.06
18	111	4,873	1.19	0.07	0.03
19	396	5,269	1.29	0.27	0.11
20	238	5,507	1.35	0.17	0.07
21	126	5,633	1.38	0.10	0.04
22	142	5,775	1.42	0.11	0.05
23	200	5,975	1.46	0.17	0.07
24	125	6,100	1.50	0.11	0.04
25	84	6,184	1.52	0.08	0.03
26	124	6,308	1.55	0.13	0.05
27	162	6,470	1.59	0.17	0.07
28	168	6,638	1.63	0.19	0.07
29	51	6,689	1.64	0.06	0.02
30	23	6,712	1.65	0.03	0.01
31	50	6,762	1.66	0.07	0.03
32	28	6,790	1.66	0.04	0.02
33	69	6,859	1.68	0.10	0.04
34	83	6,942	1.70	0.13	0.05
35	88	7,030	1.72	0.15	0.06
				Mean 0.12	Mean 0.05

^{125}I labelled Albumin / ^{51}Cr labelled Erythrocytes Experiment in Normal and Fluke-Infected Rabbits
Phase 2. Day 35 - 70 Post Infection
Daily Whole Blood and Red Cell Clearances

Rabbit No. 199 - Control

Day of Expt.	Faecal Activity	Cumulative Activity	Cumulative Activity as % Inj.	Whole Blood Clearance (ml)	R.B.C. Clearance (ml)
0	13				
1	190	203	0.05	0.03	0.01
2	280	483	0.11	0.05	0.02
3	211	694	0.16	0.04	0.02
4	130	824	0.19	0.03	0.01
5	140	964	0.22	0.03	0.01
6	114	1,078	0.25	0.03	0.01
7	170	1,248	0.29	0.04	0.02
8	77	1,325	0.31	0.02	0.01
9	86	1,411	0.33	0.02	0.01
10	74	1,485	0.35	0.02	0.01
11	105	1,590	0.37	0.03	0.01
12	134	1,724	0.40	0.04	0.02
13	110	1,834	0.43	0.04	0.01
14	88	1,922	0.45	0.03	0.01
15	100	2,022	0.47	0.04	0.01
16	84	2,106	0.49	0.03	0.01
17	80	2,186	0.51	0.03	0.01
18	73	2,259	0.53	0.03	0.01
19	73	2,332	0.54	0.03	0.01
20	88	2,420	0.56	0.04	0.02
21	83	2,503	0.58	0.04	0.02
22	112	2,615	0.61	0.06	0.02
23	112	2,727	0.63	0.06	0.02
24	97	2,824	0.66	0.06	0.02
25	81	2,905	0.68	0.05	0.02
26	68	2,973	0.69	0.04	0.02
27	215	3,188	0.74	0.13	0.06
28	98	3,286	0.76	0.07	0.03
29					
30	116	3,402	0.79	0.10	0.04
31	208	3,610	0.84	0.19	0.07
32	96	3,706	0.86	0.09	0.03
33	70	3,776	0.87	0.07	0.01
34	56	3,832	0.89	0.06	0.02
35	66	3,898	0.91	0.08	0.03
				Mean 0.05	Mean 0.02

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment in Normal and Fluke Infected Rabbits
Phase 2. Day 35 - 70 Post Infection

Rabbit No. 312 - Control

Day of Expt.	Faecal Activity	Cumulative Activity	Cumulative Activity as % Inj.	Whole Blood Clearance (ml)	R.B.C. Clearance (ml)
0	16				
1	168	184	0.05	0.03	0.01
2	210	394	0.10	0.05	0.02
3	249	643	0.16	0.06	0.02
4	112	755	0.19	0.03	0.01
5	158	913	0.23	0.04	0.02
6	178	1,091	0.28	0.05	0.02
7	134	1,225	0.31	0.04	0.02
8	185	1,410	0.36	0.06	0.02
9	110	1,520	0.38	0.04	0.01
10	95	1,615	0.41	0.03	0.01
11	98	1,713	0.43	0.03	0.01
12	203	1,916	0.48	0.07	0.03
13	257	2,173	0.55	0.10	0.04
14	275	2,448	0.62	0.11	0.04
15	265	2,713	0.69	0.12	0.05
16	200	2,913	0.74	0.09	0.04
17	126	3,039	0.77	0.06	0.02
18	123	3,162	0.80	0.06	0.02
19	155	3,317	0.84	0.08	0.03
20	182	3,499	0.88	0.10	0.04
21	162	3,661	0.93	0.09	0.04
22	113	3,774	0.95	0.07	0.03
23	84	3,858	0.98	0.06	0.02
24	125	3,983	1.00	0.09	0.03
25	97	4,080	1.03	0.07	0.03
26	81	4,161	1.05	0.06	0.02
27	168	4,329	1.09	0.14	0.05
28	158	4,487	1.13	0.14	0.05
29	107	4,594	1.16	0.10	0.04
30	192	4,786	1.21	0.19	0.07
31	160	4,946	1.25	0.17	0.06
32	108	5,054	1.28	0.12	0.05
33	92	5,146	1.30	0.11	0.04
34	83	5,229	1.32	0.10	0.04
35	112	5,341	1.35	0.14	0.06
				Mean 0.08	Mean 0.03

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes in Normal and Fluke-infected Rabbits
Phase 2. Day 35 - 70 Post Infection
Daily Whole Blood and Red Cell Clearances

Rabbit No. 304 - Infected

Day of Experiment	Days Post Infection	Faecal Activity	Cumulative Activity	Cumulative Activity as % Inj.	Whole Blood Clearance (ml)	R.B.C. Clearance (ml)
0	35	56				
1	36	275	331	0.09	0.06	0.02
2	37	252	583	0.16	0.06	0.02
3	38	203	786	0.22	0.05	0.02
4	39	261	1,047	0.29	0.06	0.03
5	40	257	1,304	0.37	0.08	0.03
6	41	316	1,620	0.45	0.11	0.04
7	42	123	1,743	0.49	0.04	0.02
8	43	340	2,083	0.58	0.13	0.05
9	44	309	2,392	0.67	0.13	0.05
10	45	403	2,795	0.78	0.13	0.07
11	46	527	3,322	0.93	0.26	0.10
12	47	537	3,859	1.08	0.29	0.11
13	48	2,755	6,614	1.85	1.61	0.60
14	49	1,533	8,147	2.28	0.95	0.36
15	50	1,184	9,331	2.61	0.81	0.30
16	51	1,354	10,685	2.99	1.00	0.37
17	52	1,186	11,871	3.33	0.93	0.35
18	53	864	12,735	3.57	0.74	0.28
19	54	706	13,441	3.77	0.66	0.24
20	55	861	14,302	4.00	0.86	0.33
21	56	942	15,244	4.27	1.00	0.39
22	57	1,260	16,504	4.62	1.52	0.56
23	58	1,271	17,775	4.98	1.74	0.60
24	59	1,393	19,168	5.37	2.11	0.73
25	60	752	19,920	5.58	1.28	0.42
26	61	948	20,868	5.85	1.77	0.62
27	62	1,127	21,995	6.16	2.40	0.81
28	63	1,179	23,174	6.49	2.84	0.97
29	64	610	23,784	6.66	1.64	0.56
30	65	819	24,603	6.89	2.47	0.86
31	66	710	25,313	7.10	2.42	0.84
32	67	634	25,947	7.27	2.40	0.84
33	68	454	26,401	7.40	1.94	0.69
34	69	680	27,081	7.59	3.24	1.14
35	70	568	27,649	7.75	2.99	1.08

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes in Normal and Fluke-infected Rabbits.
Phase 2. Day 35 - 70 Post-Infection
Daily Whole Blood and Red Cell Clearances

Rabbit No. 307 - Infected

Day of Experiment	Days Post Infection	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Ini.	Whole Blood Clearance (ml)	R.B.C. Clearance (ml)
0	35	185				
1	36	872	1,057	0.21	0.16	0.06
2	37	688	1,745	0.35	0.14	0.05
3	38	775	2,520	0.50	0.17	0.06
4	39	801	3,321	0.66	0.20	0.07
5	40	1,212	4,533	0.91	0.33	0.11
6	41	1,519	6,052	1.21	0.46	0.13
7	42	1,148	7,200	1.44	0.38	0.13
8	43	1,514	8,714	1.74	0.57	0.18
9	44	1,334	10,048	2.00	0.55	0.18
10	45	2,525	12,573	2.51	1.14	0.36
11	46	2,015	14,588	2.91	1.01	0.32
12	47	3,732	18,320	3.66	2.05	0.66
13	48	2,589	20,909	4.18	1.57	0.50
14	49	6,377	27,286	5.45	4.31	1.35
15	50	2,695	29,981	5.99	1.97	0.62
16	51	4,450	34,431	6.88	3.73	1.13
17	52	4,746	39,177	7.83	4.39	1.43
18	53	3,571	42,748	8.54	3.92	1.26
19	54	4,622	47,370	9.46	6.25	1.88
20	55	2,356	49,716	9.93	3.75	1.10
21	56	3,981	53,697	10,73	7.78	2.19
22	57	2,532	56,229	11.23	5.94	1.65
23	58	4,550	60,779	12.14	12.88	3.69
24	59	1,354	62,133	12.41	4.77	1.34
25	60	1,528	63,661	12.72	5.39	1.83
26	61	3,091	66,752	13.34	16.00	4.55
27	62	1,490	68,242	13.63	9.37	2.69
28	63	1,549	69,791	13.94	11.82	2.80
29	64	824	70,615	14.11	7.63	2.27
30	65	1,238	71,853	14.36	13.60	4.13
31	66	1,107	72,960	14.58	14.95	4.67
32	67	924	73,884	14.76	16.21	4.69
33	68	272	74,156	14.82	6.48	1.72
34	69	820	74,976	14.98	24.10	6.03
35	70	576	75,552	15.09	25.00	6.55

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment in Normal and Fluke-infected Rabbits
Phase 2. Day 35 - 70 Post Infection
Daily Whole Blood and Red Cell Clearances

Rabbit No. 308 - Infected

Day of Expt.	Day Post Infection	Faecal Activity	Cumulative Activity	Cumulative as % Injected	Whole Blood Clearance (ml)	R.B.C. Clearance (ml)
0	35	2				
1	36	126	128	0.03	0.02	0.01
2	37	156	284	0.07	0.03	0. 01
3	38	271	555	0.14	0.06	0.02
4	39	90	645	0.16	0.02	0.01
5	40	221	866	0.22	0.06	0.02
6	41	55	921	0.23	0.02	0.01
7	42	12	933	0.24	0.004	0.001
8	43	557	1,490	0.38	0.18	0.07
9	44	271	1,761	0.45	0.09	0.03
10	45	965	2,726	0.69	0.36	0.13
11	46	854	3,580	0.91	0.24	0.13
12	47	1,779	5,359	1.36	0.75	0.27
13	48	1,406	6,765	1.71	0.64	0.24
14	49	896	7,661	1.94	0.44	0.16
15	50	1,052	8,713	2.20	0.54	0.21
16	51	1,219	9,932	2.51	0.69	0.25
17	52	867	10,799	2.73	0.52	0.19
18	53	1,555	12,354	3.13	1.00	0.38
19	54	1,426	13,780	3.49	0.98	0.37
20	55	1,341	15,121	3.82	1.00	0.37
21	56	441	15,562	3.94	0.35	0.13
22	57	2,099	17,661	4.47	1.77	0.66
23	58	1,398	19,159	4.85	1.30	0.47
24	59	1,329	20,488	5.18	1.30	0.48
25	60	1,519	22,007	5.57	1.61	0.59
26	61	1,714	23,721	6.00	2.05	0.71
27	62	1,886	25,607	6.48	2.50	0.85
28	63	3,415	29,023	7.34	5.08	1.65
29	64	1,814	30,837	7.80	2.93	0.97
30	65	791	31,628	8.00	1.40	0.48
31	66	2,099	33,727	8.53	4.23	1.45
32	67	1,843	35,570	9.00	4.12	1.39
33	68	2,153	37,723	9.54	5.33	1.81
34	69	1,408	39,131	9.90	3.90	1.34
35	70	2,140	41,271	10.44	6.50	2.28

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment in Normal and Fluke-infected Rabbits

Phase 2. Day 30 - 70 Post Infection

Daily Whole Blood and Red Cell Clearances

Rabbit No. 310 - Infected

Day of Expt.	Day Post Infection	Faecal Activity	Cumulative Activity	Cumulative as % Injected	Whole Blood Clearance (ml)	R.B.C. Clearance (ml)
0	35					
1	36					
2	37	356	356	0.06	0.05	0.02
3	38	752	1,108	0.19	0.11	0.04
4	39	2,760	3,868	0.66	0.44	0.16
5	40	1,817	5,685	0.98	0.32	0.12
6	41	1,694	7,379	1.27	0.32	0.12
7	42	2,074	9,453	1.62	0.42	0.15
8	43	1,600	11,053	1.90	0.25	0.13
9	44	2,416	13,469	2.31	0.57	0.21
10	45	2,707	16,176	2.78	0.69	0.25
11	46	5,904	22,080	3.79	1.65	0.61
12	47	3,012	25,092	4.31	0.91	0.34
13	48	3,356	28,448	4.89	1.10	0.42
14	49	3,643	32,091	5.51	1.27	0.48
15	50	1,958	34,049	5.85	0.75	0.28
16	51	5,698	39,747	6.83	2.32	0.85
17	52	2,932	42,679	7.33	1.53	0.50
18	53	5,490	48,169	8.28	2.82	1.00
19	54	5,715	53,884	9.26	3.55	1.13
20	55	755	54,639	9.39	0.56	0.16
21	56	5,760	60,399	10.38	5.04	1.36
22	57	2,993	63,392	10.89	3.07	0.87
23	58	3,907	67,299	11.56	4.85	1.29
24	59	2,644	69,943	12.01	3.91	1.09
25	60	2,110	72,053	12.38	3.66	1.00
26	61	1,992	74,045	12.72	4.20	1.12
27	62	2,970	77,015	13.23	7.32	2.05
28	63	4,627	81,642	14.02	13.64	3.82
29	64	938	82,580	14.19	3.26	0.93
30	65	1,647	84,227	14.47	6.95	1.94
31	66	2,868	87,095	14.96	14.13	3.95
32	67	2,077	89,172	15.32	12.29	3.43
33	68	1,247	90,419	15.53	9.24	2.47
34	69	670	91,089	15.65	5.63	1.56
35	70	1,670	92,759	15.94	16.37	4.42

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment
in Normal and Fluke-Infected Rabbits
Phase 2. Day 35 - 70 Post Infection
Daily Whole Blood and Red Cell Clearances

Rabbit No. 311 - Infected

Day of Expt.	Day Post Infection	Faecal Activity	Cumulative Activity	Cumulative as % Injected	Whole Blood Clearance (ml)	R.B.C. Clearance (ml)
0	35	54				
1	36	155	209	0.04	0.03	0.01
2	37	770	979	0.21	0.14	0.05
3	38	91	1,070	0.23	0.02	0.01
4	39	158	1,228	0.26	0.03	0.01
5	40	1,135	2,363	0.50	0.26	0.09
6	41	2,755	5,118	1.09	0.69	0.26
7	42	1,575	6,693	1.43	0.42	0.16
8	43	290	6,983	1.49	0.09	0.03
9	44	2,106	9,089	1.94	0.68	0.25
10	45	2,393	11,482	2.45	0.83	0.30
11	46	2,699	14,181	3.02	1.03	0.36
12	47	4,768	18,949	4.03	1.97	0.69
13	48	3,912	22,861	4.87	1.74	0.60
14	49	2,263	25,124	5.35	1.09	0.37
15	50	614	25,738	5.49	0.32	0.11
16	51	2,649	28,387	6.05	1.51	0.51
17	52	4,062	32,449	6.92	2.50	0.84
18	53	5,083	37,532	8.00	3.43	1.11
19	54	4,430	41,962	8.94	3.21	1.09
20	55	3,612	45,574	9.70	2.91	0.90
21	56	1,658	47,232	10.06	1.41	0.44
22	57	4,000	51,232	10.92	3.74	1.16
23	58	2,310	53,542	11.41	2.39	0.71
24	59	2,752	56,294	12.00	3.07	0.92
25	60	965	57,259	12.20	1.17	0.34
26	61	2,781	60,040	12.80	3.84	1.15
27	62	3,322	63,362	13.51	5.66	1.55
28	63	3,740	67,102	14.30	7.42	2.00
29	64	3,673	70,775	15.09	8.58	2.24
30	65	3,990	74,765	15.94	10.90	2.78
31	66	1,960	76,725	16.35	6.30	1.59
32	67	2,823	79,548	16.95	10.77	2.61
33	68	1,486	81,034	17.27	6.72	1.59
34	69	1,744	82,778	17.64	9.04	2.12
35	70	1,674	84,452	18.00	10.08	2.29

^{125}I-LABELLED ALBUMIN/ ^{51}Cr-LABELLED ERYTHROCYTES EXPERIMENTS

PHASE 3:-EXPERIMENTAL RESULTS

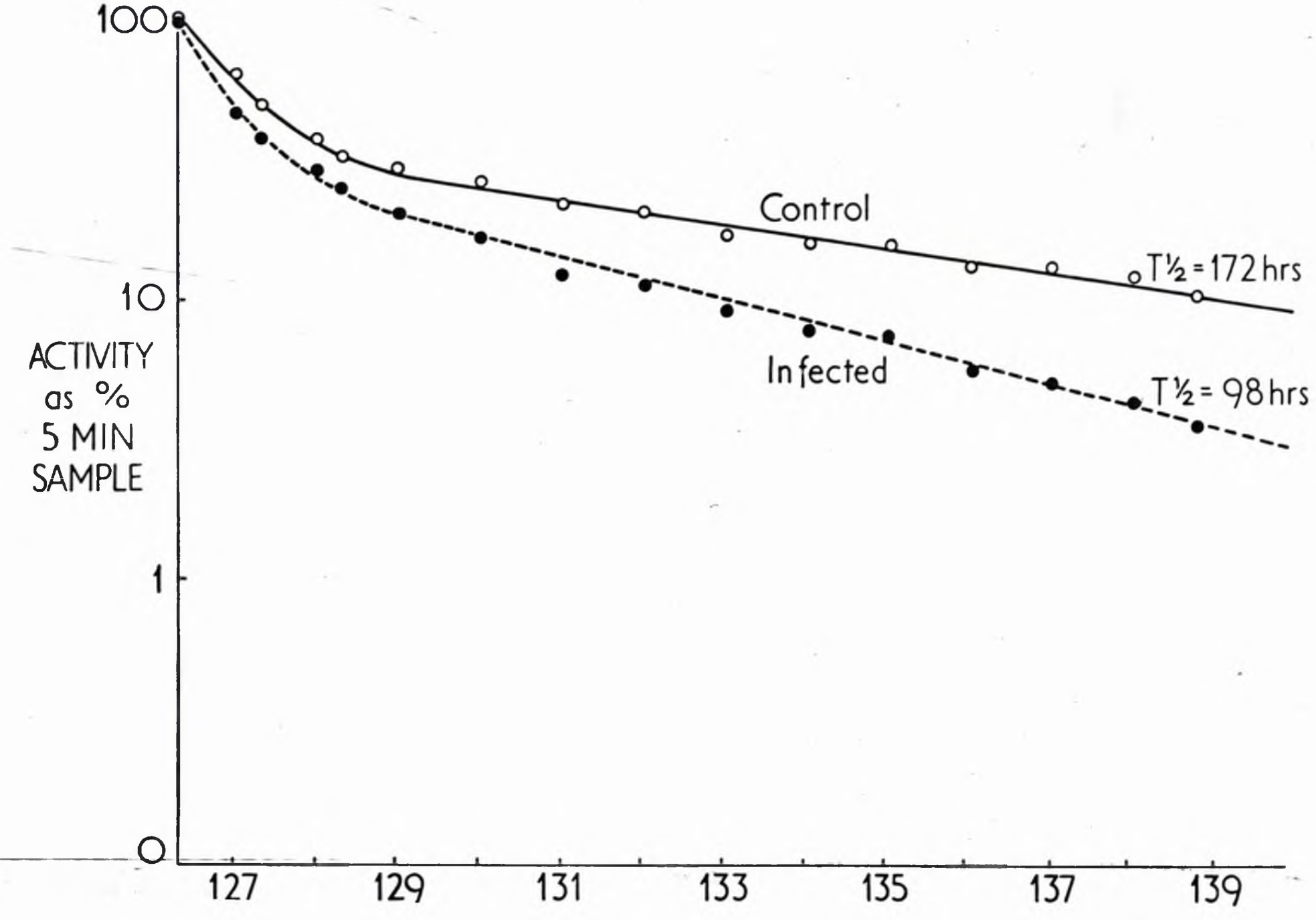
^{125}I-labelled Albumin / ^{51}Cr-labelled Erythrocytes Experiment in Normal and Fluke Infected Rabbits

Phase 3

Packed Cell Volumes and Serum Protein Data

	Rabbit No.	P.C.V.	Total Protein (G/%)	Albumin (g/%)	Globulin (g/%)	A/G	Globulin (g/%)		
							Alpha	Beta	Gamma
CONTROL	199	40	5.80	2.90	2.90	1.00	0.50	1.54	1.09
	312	37	6.30	3.23	3.07	1.05	0.44	1.27	1.30
	317	41	5.80	3.10	2.70	1.15	0.27	1.18	1.08
	Mean	39	5.97	3.08	2.89	1.07	0.40	1.33	1.16
	S.D.	2.08	0.29	0.17	0.18	0.08	0.12	0.19	0.12
	P 2/3	>0.10	>0.10	>0.10	>0.10	>0.10	>0.10	>0.10	>0.10
INFECTED	304	29	5.00	2.50	2.50	1.00	0.45	1.36	0.68
	308	29	5.72	2.64	3.08	0.86	0.44	1.70	0.91
	310	15	6.51	2.73	3.78	0.72	0.69	2.06	1.03
	311	21	5.73	2.51	3.22	0.78	0.52	1.70	0.99
	Mean	24	5.74	2.60	3.15	0.84	0.53	1.71	0.90
	S.D.	6.80	0.62	0.11	0.53	0.12	0.12	0.29	0.16
	P	<0.02	>0.10	<0.10	>0.10	<0.05	>0.10	>0.10	<0.10
	P 2/3	<0.01	<0.02	>0.10	<0.02	<0.02	<0.01	>0.10	>0.05

^{125}I-LABELLED ALBUMIN DISAPPEARANCE CURVE FOLLOWING INFECTION with F HEPATICA. PHASE 3 DAY 126 - 140 POST-INFECTION.



^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiments in Rabbits Following Infection with F. hepatica
Phase 3. 18 - 20 Weeks Post Infection
^{125}I Labelled Albumin - Experimental Results

	I N F E C T E D				C O N T R O L		
	304	308	310	311	317	199	312
Plasma Volume (ml/Kg)	31.2	40.5	37.1	40.0	40.0	36.4	30.1
CA (gm/Kg)	0.780	1.069	1.013	1.004	1.241	1.056	0.972
TA (Sterling, gm/Kg)	2.080	3.341	3.172	3.710	2.976	2.514	3.316
EA (Sterling, gm/Kg)	1.300	2.272	2.159	2.696	1.735	1.458	2.344
TA (Campbell, gm/Kg)	1.900	3.080	2.870	3.010	2.550	2.173	2.702
EA (Campbell, gm/Kg)	1.120	2.021	1.857	2.006	1.309	1.117	1.730
EA/CA (Sterling)	1.67	2.13	2.13	2.70	1.40	1.38	2.41
EA/CA (Campbell)	1.44	1.89	1.83	2.00	1.05	1.06	1.78
EA/TA (Sterling)	0.63	0.68	0.68	0.73	0.58	0.58	0.71
EA/TA (Campbell)	0.60	0.65	0.65	0.67	0.51	0.51	0.64
$T\frac{1}{2}$ (hours)	101	99	73	117	184	172	159
T Equil. (hours)	39	40	30	50	58	59	34
F (CA) (K - Campbell)	0.375	0.411	0.571	0.402	0.154	0.207	0.313
K Matthews	0.385	0.442	0.606	0.411	0.195	0.205	0.314
F (TA)	0.157	0.127	0.142	0.126	0.080	0.117	0.107
Plasma clearance (ml/24hrs)	1.02	1.20	2.95	0.97	0.27	0.34	0.36
Absolute Amount Albumin Catabolised /gm/Kg/ 24 hrs)	0.293	0.439	0.578	0.404	0.191	0.219	0.304

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment in Normal and Fluke Infected Rabbits
Phase 3. 18 - 20 Weeks post Infection
Daily Plasma Clearances

Day of Expt.	Days post Infection	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Plasma Clearance (ml)
0	126	878			0.05
1	127	4,838	5,716	0.33	0.39
2	128	3,872	9,588	0.55	0.47
3	129	792	10,380	0.60	0.13
4	130	116	10,496	0.60	0.02
5	131	1,124	11,620	0.67	0.23
6	132	2,612	14,238	0.82	0.58
7	133	1,607	15,845	0.91	0.39
8	134	991	16,836	0.97	0.26
9	135	640	17,476	1.01	0.19
10	136	680	18,156	1.05	0.22
11	137	370	18,526	1.07	0.13
12	138	612	19,138	1.10	0.23
13	139	521	19,659	1.13	0.22

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment in Normal and Fluke Infected Rabbits
Phase 3. 18 - 20 Weeks Post Infection
Daily Plasma Clearances

Rabbit No. 199

Day of Expt.	Days Post Infection	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Plasma Clearance (ml)
0	126	2,787			0.13
1	127	6,780	9,567	0.60	0.47
2	128	4,637	14,204	0.89	0.50
3	129	3,292	17,496	1.10	0.46
4	130	2,621	20,117	1.26	0.42
5	131	1,570	21,687	1.36	0.28
6	132	668	22,355	1.40	0.13
7	133	2,128	24,483	1.53	0.46
8	134	1,226	26,709	1.61	0.29
9	135	1,253	26,962	1.69	0.33
10	136	1,040	28,002	1.75	0.30
11	137	824	28,826	1.81	0.26
12	138	952	29,778	1.87	0.33
13	139	336	30,114	1.89	0.13

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment in Normal and Fluke-Infected Rabbits
Phase 3. 18 - 20 Weeks Post Infection
Daily Plasma Clearances

Rabbit No. 312

Day of Expt.	Days Post Infection	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Plasma Clearance (ml)
0	126	3,989			0.15
1	127	1,960	5,949	0.34	0.17
2	128	2,831	8,780	0.51	0.39
3	129	1,728	10,508	0.61	0.30
4	130	2,152	12,660	0.73	0.41
5	131	1,030	13,690	0.79	0.22
6	132	1,380	15,070	0.87	0.33
7	133	1,899	16,959	0.98	0.51
8	134	1,200	18,159	1.05	0.36
9	135	845	19,004	1.10	0.27
10	136	1,555	20,559	1.19	0.57
11	137	988	21,547	1.24	0.40
12	138	737	22,284	1.29	0.34
13	139	540	22,824	1.32	0.27

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment in Normal and Fluke Infected Rabbits
phase 3. 18 - 20 Weeks Post Infection
Daily Plasma Clearances

Rabbit No. 304

Day of Expt.	Days Post Infection	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Plasma Clearance (ml)
0	126	6,516			0.34
1	127	3,732	10,248	0.68	0.41
2	128	3,783	14,031	0.93	0.67
3	129	3,500	17,531	1.16	0.77
4	130	3,165	20,696	1.38	0.84
5	131	5,602	26,298	1.75	1.74
6	132	2,305	28,603	1.90	0.84
7	133	1,979	30,582	2.03	0.86
8	134	1,648	32,230	2.14	0.84
9	135	1,396	33,626	2.23	0.84
10	136	1,779	35,405	2.35	1.27
11	137	1,668	37,073	2.46	1.40
12	138	1,253	38,326	2.55	1.25
13	139	804	39,130	2.60	0.94

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment in Normal and Fluke Infected Rabbits
Phase 3. 18 - 20 Weeks Post Infection
Daily Plasma Clearance

Rabbit No. 308

Day of Expt.	Days Post Infection	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Plasma Clearance (ml)
0	126	4,646			0.22
1	127	9,355	14,001	0.82	0.87
2	128	5,488	19,489	1.14	0.90
3	129	5,820	25,309	1.47	1.34
4	130	1,957	27,266	1.59	0.59
5	131	6,090	33,356	1.94	2.27
6	132	2,514	35,870	2.09	1.11
7	133	1,917	37,787	2.20	1.00
8	134	2,330	40,117	2.34	1.45
9	135	1,760	41,877	2.44	1.33
10	136	1,319	43,196	2.52	1.14
11	137	969	44,165	2.57	0.98
12	138	1,240	45,405	2.65	1.50
13	139	756	46,161	2.69	1.08

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment in Normal and Fluke Infected Rabbits
Phase 3. 18 - 20 Weeks Post Infection
Daily Plasma Clearances

Rabbit No. 310

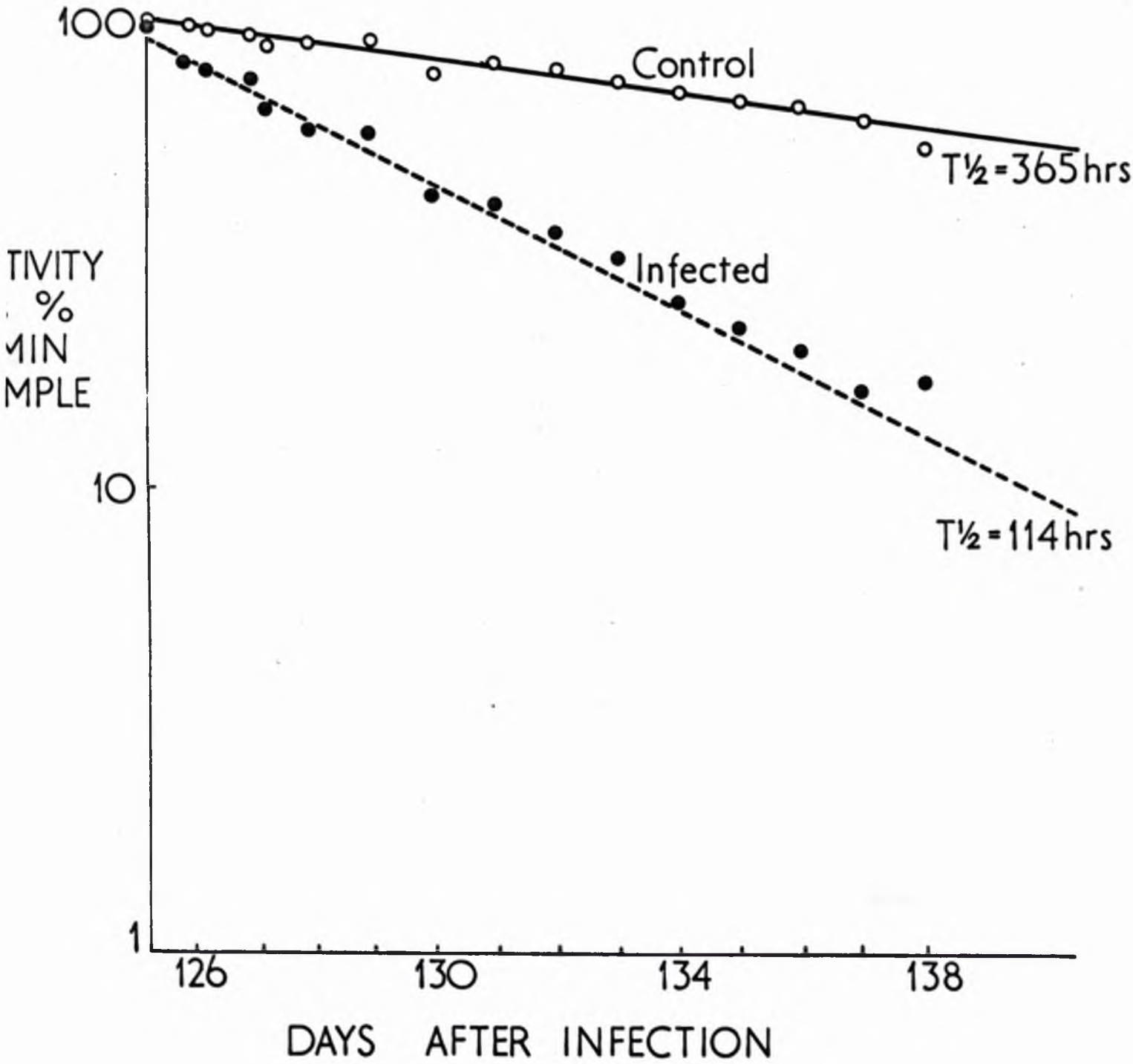
Day of Expt.	Days Post Infection	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Plasma Clearance (ml)
0	126	10,845			0.37
1	127	13,057	23,902	1.27	1.21
2	128	11,763	35,665	1.89	1.80
3	129	11,007	46,472	2.47	2.19
4	130	13,381	60,053	3.18	3.34
5	131	11,187	71,240	3.77	3.50
6	132	8,710	79,950	4.24	3.40
7	133	12,685	92,635	4.91	6.23
8	134	5,184	97,819	5.18	3.18
9	135	5,011	102,830	5.44	3.83
10	136	2,832	105,662	5.60	2.70
11	137	2184	107,846	5.71	2.59
12	138	1,276	109,122	5.78	1.91
13	139	1,305	110,427	5.85	2.43

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiment in Normal and Fluke Infected Rabbits
Phase 3. 18 - 20 Weeks Post Infection
Daily Plasma Clearances

Rabbit No. 311

Day of Expt.	Days Post Infection	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Plasma Clearance (ml)
0	126	6,601			0.37
1	127	4,990	11,591	0.89	0.57
2	128	5,904	17,495	1.34	1.17
3	129	4,743	22,238	1.70	1.34
4	130	1,767	24,005	1.84	0.63
5	131	1,256	25,261	1.93	0.52
6	132	1,201	26,462	2.03	0.57
7	133	4,433	30,895	2.37	2.42
8	134	1,738	32,633	2.50	1.09
9	135	1,184	33,817	2.59	0.86
10	136	753	34,570	2.65	0.63
11	137	738	35,308	2.70	0.71
12	138	941	36,249	2.78	1.05
13	189	770	37,019	2.83	0.99

^{51}Cr-LABELLED ERYTHROCYTES - R.B.C. ACTIVITY FOLLOWING INFECTION with F. HEPATICA. PHASE 3 DAY 126-140 POST INFECTION



^{125}I Labelled Albumin and ^{51}Cr Labelled Erythrocytes Experiments in Rabbits Following Infection with F. hepatica

Phase 3. 18 - 20 Weeks post-infection

^{51}Cr Labelled Erythrocytes - Experimental Results

	Rabbit No.	Blood Volume (ml)	Blood Volume (ml/Kg)	Circulating Red Cell Volume (ml)	Circulating Red Cell Volume (ml/Kg)	Whole Blood $T\frac{1}{2}$ (hrs)	Red Cell $T\frac{1}{2}$ (hrs)	"Faecal Clearance" Whole Blood (ml/24hrs)	"Faecal Clearance" R.B.C. (ml/24hrs)	Flukes Recovered
CONTROL	317	124.62	53.38	30.02	13.34	307	442	0.06	0.02	
	199	99.65	50.01	25.05	12.22	238	324	0.04	0.01	
	312	94.07	43.71	29.27	13.61	345	328	0.06	0.02	
	Mean	106.11	49.03	28.11	13.06	296.7	364.7	0.053	0.017	
	S.D.	16.27	4.91	2.68	0.74	54.2	67.0	0.01	-	
INFECTED	304	103.1	40.45	23.60	9.25	179	181	5.49	1.59	10
	308	107.56	52.48	24.56	11.98	120	126	9.38	2.42	16
	310	77.71	44.41	12.79	7.31	85	70	13.59	2.82	26
	311	91.46	49.44	17.46	9.44	71	79	21.08	3.51	27
	Mean	94.96	46.70	19.60	9.50	113.75	11.40	12.39	2.59	
	S.D.	13.35	5.33	5.52	1.92	48.13	50.97	6.67	0.80	
	P	>0.10	>0.10	>0.05	<0.05	<0.01	<0.01	<0.05	<0.01	

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiments in Normal and Fluke Infected Rabbits
Phase 3. 18 - 20 Weeks Post Infection
Daily Whole Blood and Red Cell Clearances

Rabbit No. 317 - Control

Day of Expt.	Faecal Activity (c/s)	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Whole Blood Clearance (ml/24hrs)	R.B.C. Clearance (ml/24hrs)
0	5			0.003	0.001
1	36	41	0.03	0.02	0.01
2	103	144	0.11	0.06	0.02
3	29	173	0.13	0.02	0.01
4	7	180	0.14	0.005	0.002
5	76	256	0.19	0.06	0.02
6	35	291	0.22	0.03	0.01
7	185	476	0.36	0.15	0.05
8	143	619	0.47	0.12	0.04
9	90	709	0.53	0.08	0.03
10	80	789	0.59	0.08	0.03
11	27	816	0.61	0.03	0.01
12	68	884	0.67	0.07	0.02
13	55	939	0.71	0.06	0.02

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiments in Normal and Fluke Infected Rabbits
Phase 3. 18 - 20 Weeks Post Infection
Daily Whole Blood and Red Cell Clearances

Rabbit No. 199 - Control

Day of Expt.	Faecal Activity (c/sec)	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Whole Blood Clearance (ml/24hrs)	R.B.C. Clearance (ml/24hrs)
0	14			0.01	0.005
1	20	34	0.04	0.01	0.005
2	34	68	0.07	0.02	0.01
3	28	96	0.10	0.02	0.01
4	39	135	0.14	0.03	0.01
5	28	163	0.17	0.03	0.01
6	15	178	0.19	0.01	0.005
7	42	220	0.23	0.04	0.01
8	26	246	0.26	0.03	0.01
9	42	288	0.30	0.05	0.02
10	40	328	0.34	0.05	0.02
11	20	348	0.36	0.03	0.01
12	88	436	0.46	0.13	0.04
13	16	452	0.47	0.03	0.01

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiments in Normal and Fluke Infected Rabbits
Phase 3. 18 - 20 Weeks Post Infection
Daily Whole Blood and Red Cell Clearances

Rabbit No. 312 - Control

Day of Expt.	Faecal Activity (c/s)	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Whole Blood Clearance (ml/24hrs)	R.B.C. Clearance (ml/24hrs)
0	30		0.00	0.03	0.01
1	26	56	0.08	0.03	0.01
2	37	93	0.14	0.05	0.02
3	20	113	0.17	0.03	0.01
4	23	136	0.20	0.03	0.01
5	34	170	0.25	0.05	0.02
6	48	218	0.33	0.08	0.03
7	81	299	0.45	0.13	0.05
8	48	347	0.52	0.08	0.03
9	28	375	0.56	0.05	0.02
10	61	436	0.65	0.12	0.04
11	23	459	0.69	0.05	0.02
12	28	487	0.73	0.06	0.02
13	26	513	0.77	0.06	0.02

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiments in Normal and Fluke Infected Rabbits
Phase 3. 18 - 20 Weeks Post Infection
Daily Whole Blood and Red Cell Clearances

Rabbit No. 304 - Infected

Days post Infection	Day of Expt.	Faecal Activity	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Whole Blood Clearance (ml/24hrs)	R.B.C. Clearance (ml/24hrs)
126	0	2,918			2.48	0.72
127	1	4,026	6,944	7.26	3.85	1.13
128	2	5,065	12,009	12.56	5.32	1.56
129	3	4,742	16,751	17.52	5.45	1.60
130	4	5,016	21,767	22.77	6.28	1.85
131	5	4,675	26,442	27.66	6.52	1.89
132	6	3,192	29,634	30.99	4.85	1.41
133	7	3,445	33,079	34.60	5.75	1.67
134	8	3,203	36,282	37.95	5.92	1.72
135	9	2,760	39,042	40.84	5.59	1.62
136	10	3,038	42,080	44.01	6.80	1.92
137	11	1,859	43,939	45.96	4.52	1.31
138	12	1,980	45,919	48.03	5.27	1.53
139	13	1,776	47,695	49.89	5.21	1.51

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiments in Normal and Fluke Infected Rabbits
Phase 3. 18 - 20 Weeks Post Infection
Daily Whole Blood and Red Cell Clearances

Rabbit No. 308 - Infected

Days post Infection	Day of Expt.	Faecal Activity (c/sec)	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Whole Blood Clearance (ml/24hrs)	R.B.C. Clearance (ml/24hr)
126	0	724			1.28	0.37
127	1	3,078	3,802	7.92	6.31	1.69
128	2	3,742	7,544	15.71	8.68	2.33
129	3	1,348	8,892	18.52	3.60	0.96
130	4	2,113	11,005	22.92	6.64	1.74
131	5	2,883	13,888	28.92	10.15	2.63
132	6	3,922	17,810	27.09	16.07	4.18
133	7	2,364	20,174	42.02	10.99	2.88
134	8	2,128	22,302	46.45	11.38	2.94
135	9	1,558	23,860	49.69	9.62	2.42
136	10	1,373	25,233	52.55	9.67	2.46
137	11	936	26,169	54.50	7.67	1.91
138	12	1,387	27,556	57.39	12.84	3.23
139	13	780	28,336	59.01	8.30	2.05

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiments in Normal and Fluke Infected Rabbits
Phase 3. 18 - 20 Weeks post Infection
Daily Whole Blood and Red Cell Clearances

Rabbit No. 310 - Infected :

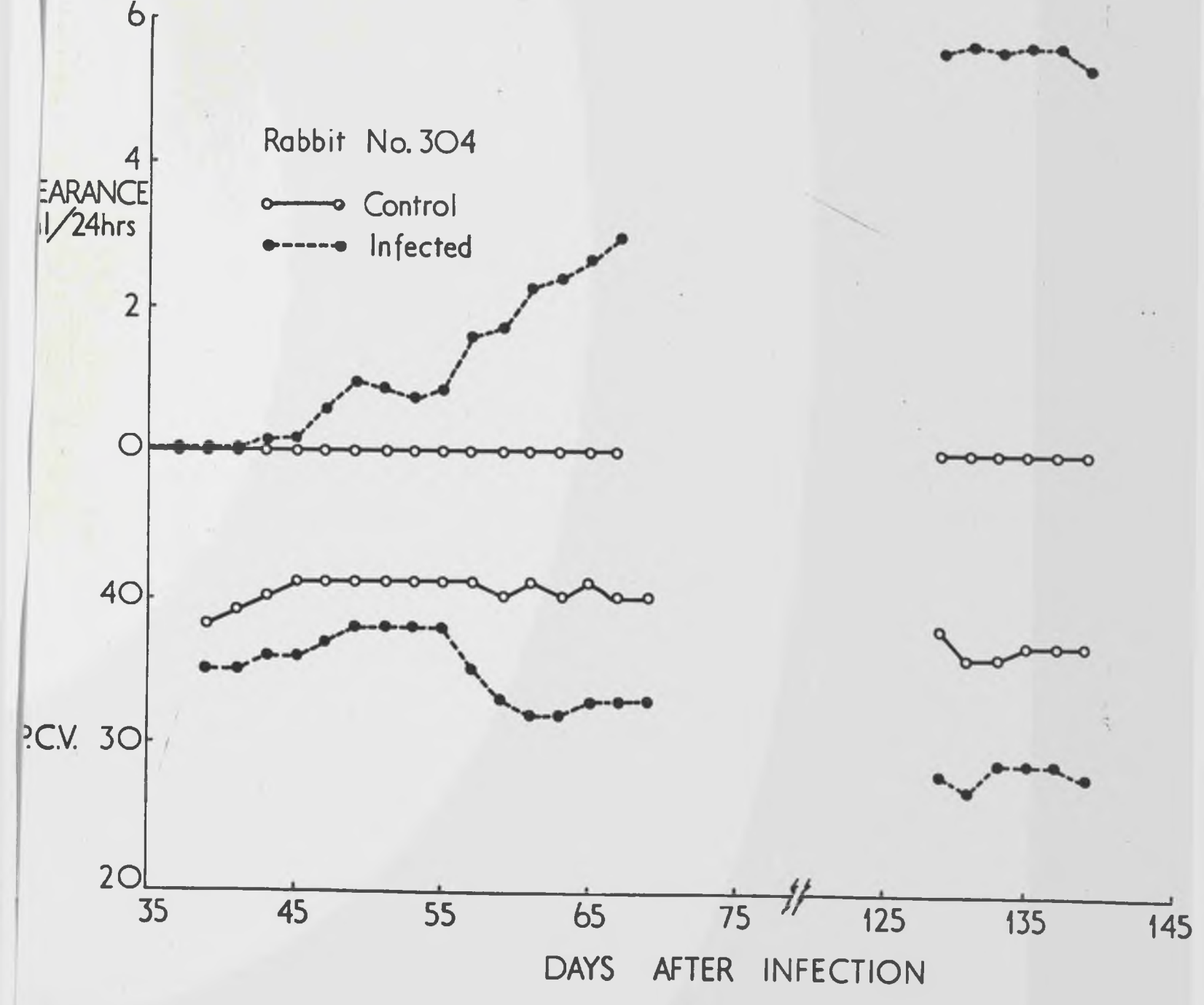
Days Post Infection	Day of Expt.	Faecal Activity (c/sec)	Cumulative Faecal Activity	Cumulative Activity as % Inj.	Whole Blood Clearance (ml/24hrs)	R.B.C. Clearance (ml/24hrs)
126	0	320			2.44	0.37
127	1	404	724	6.48	4.30	0.68
218	2	1,009	1,733	15.52	13.10	2.14
129	3	696	2,429	21.75	11.05	1.86
130	4	650	3,079	27.57	12.50	2.19
131	5	528	3,607	32.38	12.28	2.24
132	6	430	4,037	36.15	12.29	2.35
133	7	507	4,544	40.69	17.48	3.52
134	8	288	4,832	43.27	12.00	2.55
135	9	336	5,168	46.28	16.80	3.65
136	10	218	5,386	48.24	13.62	3.03
137	11	150	5,536	49.58	11.54	2.63
138	12	164	5,700	51.04	14.91	3.64
139	13	140	5,840	52.30	15 .56	4.00

^{125}I Labelled Albumin / ^{51}Cr Labelled Erythrocytes Experiments in Normal and Fluke Infected Rabbits
Phase 3. 18 - 20 Weeks Post Infection
Daily Whole Blood and Red Cell Clearances

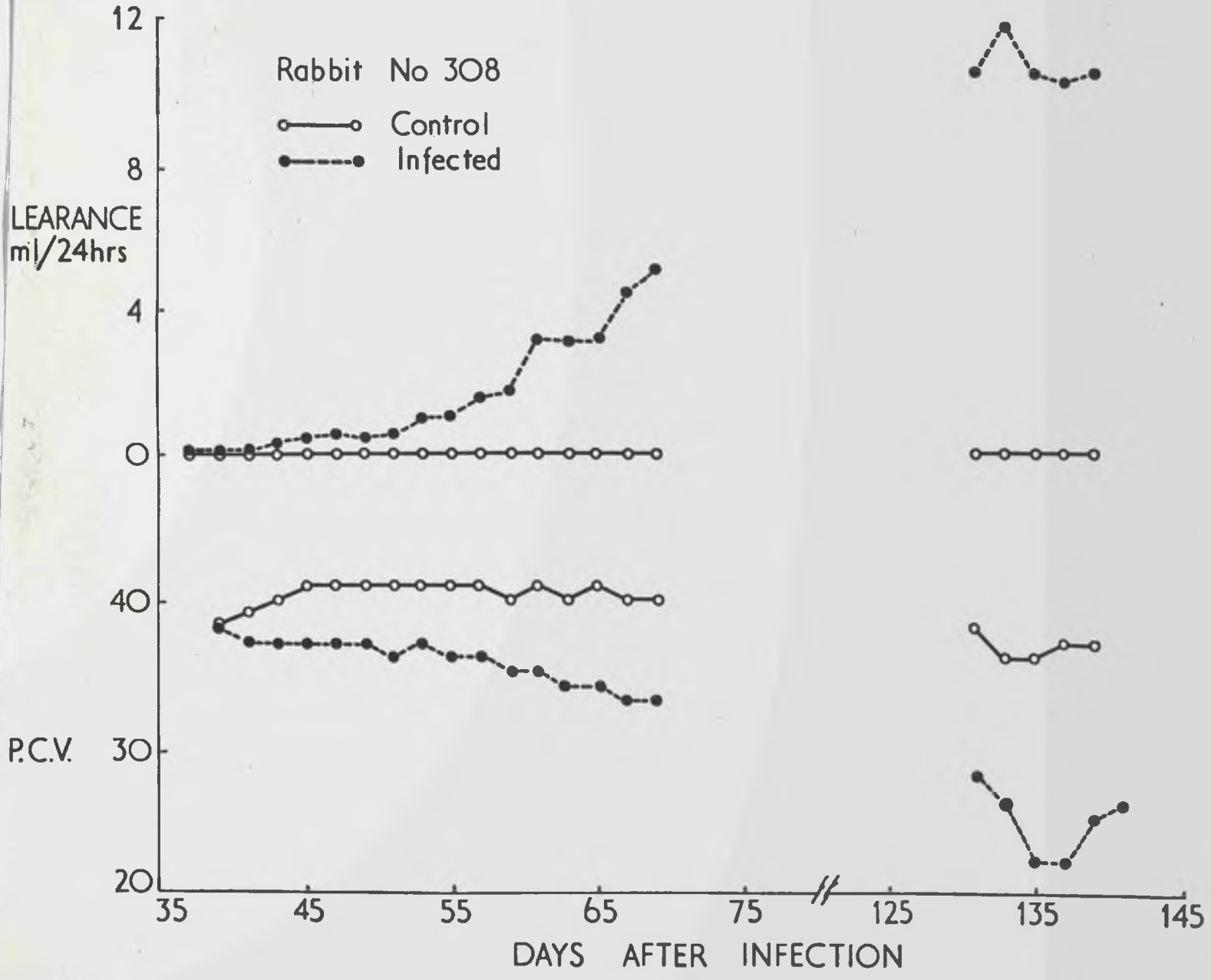
Rabbit No. 311 - Infected

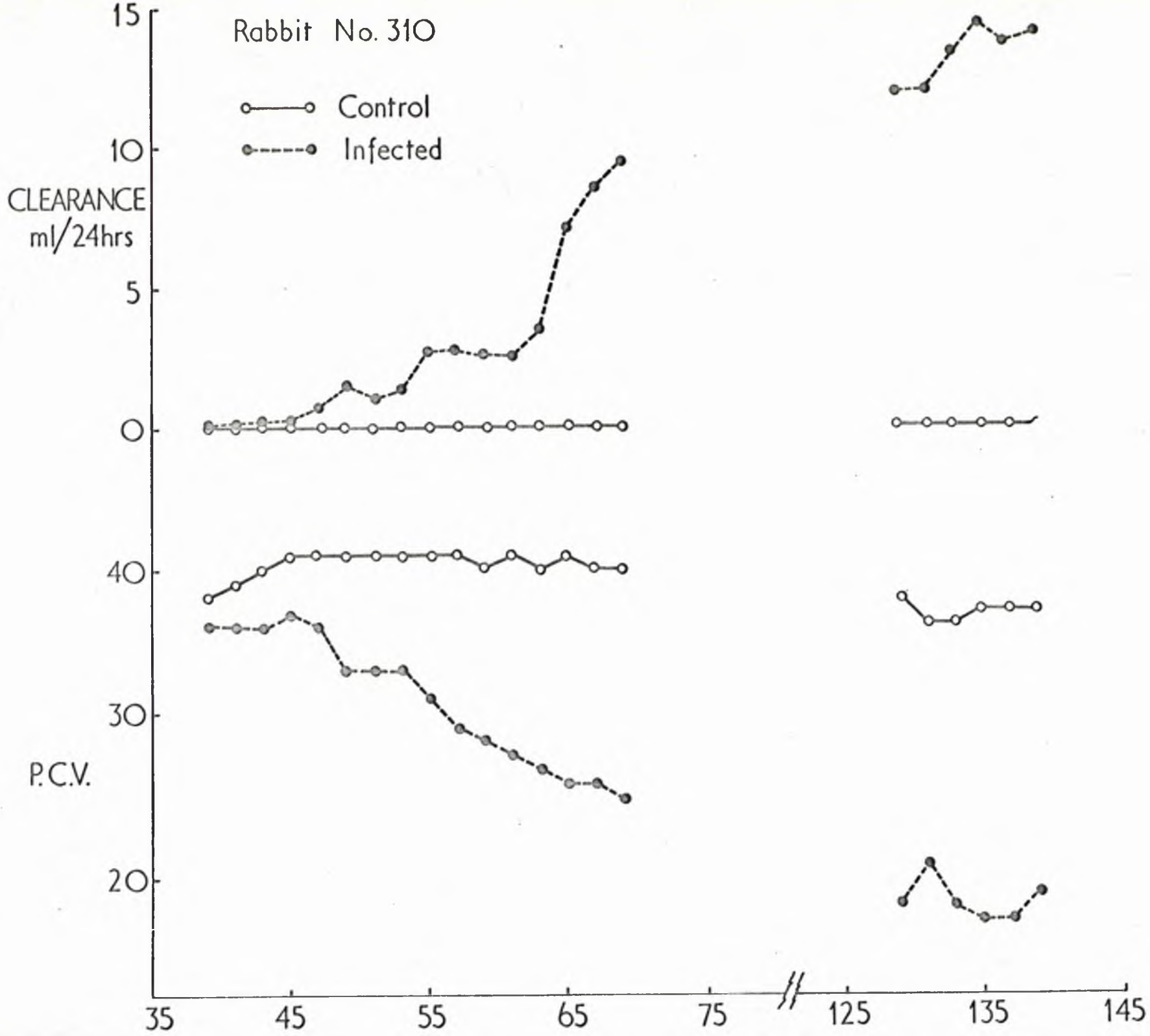
Days Post Infection	Day of Expt.	Faecal Activity (c/s)	Cumulative Faecal Activity	Cumulative Activity as % Inj	Whole Blood Clearance (ml/24hrs)	R.B.C. Clearance (ml/24hr)
126	0	1,624			3.39	0.78
127	1	1,394	3,018	8,30	4.16	0.83
128	2	4,737	7,755	21.33	17.35	3.45
219	3	3,848	11,603	31.90	18.24	3.49
130	4	1,980	13,583	37,40	11.79	2.21
131	5	1,277	14,860	40,86	9.53	1.75
132	6	1,123	15,983	43.95	10.70	1.54
133	7	1,993	17,976	49.43	24.60	4.16
134	8	1,758	19,734	54,27	27.00	4.57
135	9	1,215	20,949	57.61	24.00	3.89
136	10	678	21,627	59.47	16.95	2.71
137	11	646	22,293	61.30	20.19	3.16
138	12	738	23,031	63.33	29.52	4.42
139	13	440	23,471	64.54	22.00	3.31

^{125}I-LABELLED ALBUMIN and ^{51}Cr-LABELLED ERYTHROCYTES P.C.V. CHANGES and BLOOD CLEARANCES FOLLOWING INFECTION with F. HEPATICA



^{125}I-LABELLED ALBUMIN and ^{51}Cr-LABELLED ERYTHROCYTES P.C.V. CHANGES and BLOOD CLEARANCES FOLLOWING INFECTION with F. HEPATICA




Rabbit No. 310
Control
Infected
CLEARANCE ml/24hrs
P.C.V.
15
10
5
0
40
30
20
35
45
55
65
75
125
135
145

FAECAL EXCRETION OF ^{131}I-LABELLED P.V.P. IN RABBITS FOLLOWING INFECTION WITH F. HEPATICA

Faecal Excretion of ^{131}I - labelled P.V.P. in Rabbits Following Infection with F. Hepatica (50 Metacercariae)

DAILY PLASMA "CLEARANCES" - DAYS 1 - 18 POST - INFECTION

Days after	INFECTED			CONTROL	
Infection	11	26	34	15	38
1	-	-	-	-	-
2	-	-	3.82	1.63	2.83
3	2.52	1.81	2.40	1.90	3.32
4	1.95	1.65	3.26	2.92	2.88
5	2.29	1.06	3.48	3.21	3.48
6	2.31	1.59	3.62	4.41	3.32
7	2.66	2.01	3.38	4.06	4.09
8	3.26	1.37	2.08	4.54	4.93
9	2.89	0.96	2.64	3.82	8.87
10	3.46	0.35	2.81	5.13	16.14
11	5.38	1.79	2.93	2.77	5.76
12	4.22	1.52	3.19	3.72	3.36
13	4.64	0.66	3.67	4.78	6.41
14	3.08	1.12	3.55	8.13	5.35
15	2.74	1.74	4.01	6.63	6.74
16	4.02	3.53	7.90	4.58	4.77
17	4.00	-	3.68	7.85	4.40
Mean	3.29	1.52	3.52	4.38	5.42

Faecal Excretion of ^{131}I - labelled P.V.P. in Rabbits Following Infection with F. Hepatica (50 Metacercariae)

DAILY PLASMA "CLEARANCES" - DAYS 18 - 38 POST - INFECTION

Days after Infection	INFECTED			CONTROL	
	29	35	37	33	36
18	-	-	-	-	-
19	-	-	-	-	-
20	2.03	4.45	2.34	3.48	8.97
21	2.82	3.42	0.73	4.15	8.49
22	4.07	3.71	5.56	3.64	8.27
23	4.96	6.69	2.04	3.17	9.77
24	5.43	4.79	7.20	2.56	8.03
25	1.89	4.93	8.19	1.51	7.71
26	2.40	3.19	6.03	3.22	9.89
27	3.69	3.81	8.34	4.93	5.62
28	1.31	4.84	10.08	1.53	8.31
29	2.14	2.12	3.17	1.43	6.35
30	2.01	3.50	11.02	4.92	7.32
31	2.84	1.81	4.84	3.34	8.06
32	1.89	1.69	10.32	3.14	6.45
33	2.28	4.71	14.58	2.48	5.12
34	3.25	3.04	15.42	9.34	10.73
35	2.63	3.56	7.42	3.62	12.48
36	2.23	4.19	8.42	9.00	7.83
37	4.50	5.72	12.23	5.09	6.96
38	6.18	6.66	9.43	10.45	7.67
Mean	3.08	3.96	7.00	4.42	7.72

Faecal Excretion of ^{131}I - labelled P.V.P. in Rabbits Following Infection with F. Hepatica (50 Metacercariae)

DAILY PLASMA "CLEARANCES" - DAYS 39 - 70 POST - INFECTION

Days after Infection	INFECTED			CONTROL	
	14	18	21	42	0127
39	-	-	-	-	-
40	-	-	-	-	-
41	-	2.1	2.0	1.78	0.6
42	7.2	3.7	1.1	1.34	2.86
43	2.4	5.0	3.5	1.33	1.02
44	3.3	1.4	8.0	1.68	1.03
45	00.7	2.1	5.2	1.74	3.52
46	8.8	2.8	5.3	1.60	1.71
47	9.7	6.4	7.2	1.71	1.26
48	14.4	3.3	4.3	2.41	1.22
49	6.1	4.8	0.8	1.99	1.46
50	5.8	4.7	6.8	1.89	1.76
51	10.6	3.9	16.9	2.12	1.02
52	12.0	6.5	8.4	2.76	1.34
53	-	6.0	10.7	2.76	1.74
54	14.7	6.3	15.1	1.93	1.69
55	10.0	8.1	10.1	3.72	1.34
56	10.0	9.5	11.7	3.74	1.98
57	15.3	4.7	10.0	2.14	2.45
58	32.8	10.1	9.7	3.51	3.55
59	30.9	10.8	Killed	4.79	4.58
60	11.3	8.2		4.23	2.40
61	38.8	13.4	Mean	1.93	1.93
62	11.8	12.3			
63	12.0	8.6			
64	24.6	15.7			
65	8.2	9.8			
66	12.5	10.3			
67	28.2	14.1			
68	16.7	12.2			
	12.8	17.6			

^{95}Nb-LABELLED ALBUMIN/ ^{51}Cr-LABELLED ERYTHROCYTES EXPERIMENTS IN RABBITS AT 6-8 and 18-19 WEEKS AFTER INFECTION WITH F. HEPATICA

^{95}Nb Labelled Albumin/ ^{51}Cr Labelled Erythrocytes

6 - 8 Weeks Post-Infection Experiment

Daily Plasma Clearances

Rabbit No. 095 - Infected

Days Post Infection	Faecal Activity	Clearance (ml)
42	1171	6.2
43	153	1.3
44	436	4.9
45	894	10.9
46	612	9.7
47	284	5.9
48	234	6.5
49	483	17.9
50	409	19.5
51	432	25.4
52	236	15.7
53	347	26.7
54	-	-
55	131	13.5
56	155	18.0
57	223	29.3
		Mean 13.2

^{95}Nb Labelled Albumin/ ^{51}Cr Labelled Erythrocytes

6 - 8 Weeks Post-Infection Experiment

Daily Plasma Clearances

Rabbit No. 093 - Infected

Days Post Infection	Faecal Activity	Clearance (ml)
42	602	1.8
43	603	4.5
44	686	6.9
45	291	3.5
46	434	6.2
47	302	5.7
48	319	6.8
49	221	6.3
50	422	13.6
51	248	8.9
52	439	17.6
53	294	12.8
54	187	8.9
55	268	14.1
56	163	9.6
57	135	8.4
		Mean 8.5

^{95}Nb Labelled Albumin/ ^{51}Cr Labelled Erythrocytes

6 - 8 Weeks Post-infection Experiment 1

Daily Plasma Clearances

Rabbit No. 83 - Infected

Days Post Infection	Faecal Activity	Clearance (ml)
42	470	2.6
43	383	4.4
44	648	6.9
45	389	4.9
46	323	3.2
47	194	4.0
48	224	5.7
49	151	6.8
50	158	7.1
51	134	7.4
52	157	11.2
53	105	9.6
54	81	8.1
55	106	11.9
56	115	15.1
57	81	11.6
		Mean 7.6

^{93}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

6 - 8 Weeks Post Infection Experiment

Daily Plasma Clearances

Rabbit No. 48 - Control

Day	Faecal Activity	Clearance (ml)
1	174	0.8
2	291	2.3
3	176	1.9
4	103	1.4
5	120	2.4
6	75	2.2
7	70	2.8
8	104	5.5
9	64	4.3
10	32	3.0
11	34	6.0
12	45	6.4
13	13	2.3
14	15	2.9
15	30	7.1
		Mean 3.4

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

6 - 8 Weeks Post-Infection Experiment

Daily Plasma Clearances

Rabbit No. 103 - Control

Day	Faecal Activity	Clearance (ml)
1	175	1.18
2	-	-
3	286	4.5
4	231	4.4
5	216	4.9
6	139	4.0
7	114	4.6
8	126	7.7
9	98	7.0
10	73	5.6
11	67	5.6
12	46	4.4
13	31	3.3
14	35	4.2
15	42	5.5
16	38	5.6
		Mean 4.5

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

6 - 8 Weeks Post Infection Experiment

Daily Plasma Clearances

Rabbit No. 56 - Control

Day	Faecal Activity	Clearances (ml)
1	238	1.8
2	304	3.9
3	189	2.6
4	-	-
5	185	4.4
6	198	6.4
7	96	4.0
8	149	8.3
9	90	6.4
10	80	7.3
		Mean 4.5

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

6 - 8 Weeks Post-Infection Experiment

Daily Whole Blood and Red Cell Clearances

Rabbit No. 095 - Infested

Days Post Infection	Faecal Activity	Whole Blood Clearance (ml)	Red Cell Clearance (ml)
42	146	0.15	0.04
43	506	0.59	0.17
44	471	0.63	0.18
45	1,152	1.74	0.50
46	954	1.64	0.47
47	603	1.18	0.35
48	592	1.31	0.39
49	1,532	3.94	1.17
50	926	2.66	0.80
51	1,521	5.05	1.49
52	988	3.70	1.12
53	1,369	5.75	1.75
54	1,782	8.53	2.61
55	754	4.08	1.30
56	851	5.25	1.61
57	1,303	9.80	2.83
		Mean 3.5	Mean 1.05

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

6 - 8 Weeks Post-Infection Experiment

Daily Whole Blood and Red Cell Clearances

Rabbit No. 088 - Infected

Days Post Infection	Faecal Activity	Whole Blood Clearance (ml)	Red Cell Clearance (ml)
42	602	0.68	0.23
43	845	1.09	0.37
44	842	1.25	0.42
45	364	0.62	0.21
46	665	1.28	0.44
47	571	1.27	0.43
48	686	1.75	0.59
49	469	1.39	0.46
50	626	2.14	0.71
51	694	2.66	0.91
52	814	3.55	1.22
53	723	3.60	1.24
54	509	2.93	0.99
55	1,246	8.25	2.80
56	734	5.67	1.94
57	707	6.20	2.13
		Mean 2.8	Mean 0.94

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

6 - 8 Weeks Post-Infection Experiment

Daily Whole Blood and Red Cell Clearances

Rabbit No. 63 - Infected

Days Post Infection	Faecal Activity	Whole Blood Clearance (ml)	Red Cell Clearance (ml)
42	210	0.21	0.08
43	615	0.65	0.26
44	187	0.22	0.09
45	288	0.36	0.14
46	378	0.52	0.21
47	264	0.39	0.16
48	352	0.57	0.23
49	281	0.49	0.20
50	176	0.34	0.13
51	168	0.35	0.14
52	515	1.15	0.46
53	369	0.90	0.36
54	302	0.82	0.32
55	404	1.16	0.46
56	482	1.50	0.60
57	529	1.78	0.71
		Mean 0.71	Mean 0.28

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

6 - 8 Weeks Post-Infection Experiment

Daily Whole Blood and Red Cell Clearances

Rabbit No. 48 - Control

Day	Faecal Activity	Whole Blood Clearance (ml)	Red Cell Clearance (ml)
1	228	0.29	0.10
2	301	0.41	0.16
3	29	0.04	0.02
4	-	-	-
5	43	0.07	0.03
6	-	-	-
7	7	0.01	0.005
8	-	-	-
9	8	0.02	0.007
10	18	0.05	0.02
11	66	0.18	0.06
12	22	0.07	0.02
13	3	0.01	0.003
14	22	0.08	0.09
15	70	0.26	0.08
		Mean 0.10	Mean 0.04

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

6 - 8 Weeks Post-Infection Experiment

Daily Whole Blood and Red Cell Clearances

Rabbit No. 103 - Control

Day	Faecal Activity	Whole Blood Clearance (ml)	Red Cell Clearance (ml)
1	70	0.11	0.03
2	-	-	-
3	64	0.11	0.04
4	-	-	-
5	173	0.34	0.11
6	70	0.15	0.05
7	94	0.21	0.07
8	25	0.06	0.02
9	73	0.19	0.06
10	10	0.03	0.01
11	-	-	-
12	55	0.18	0.05
13	218	0.73	0.26
14	60	0.22	0.08
15	142	0.55	0.20
16	68	0.28	0.11
		Mean 0.20	Mean 0.07

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

6 - 8 Weeks Post-Infection Experiment

Daily Whole Blood and Red Cell Clearances

Rabbit No. 56 - Control

Day	Faecal Activity	Whole Blood Clearance (ml)	Red Cell Clearance (ml)
1	-	-	-
2	224	0.37	0.14
3	63	0.11	0.04
4	-	-	-
5	15	0.03	0.01
6	218	0.49	0.19
7	32	0.08	0.03
8	-	-	-
9	365	1.06	0.41
10	-	-	-
		Mean 0.21	Mean 0.08

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

18 - 19 Weeks Post Infection Experiment

Daily Plasma Clearances

Rabbit No. 095 - Infected

Day	Faecal Activity	Clearance (ml)
1	894	13.5
2	1,264	26.9
3	680	19.4
4	892	35.7
5	468	26.0
6	426	32.8
7	215	21.5
8	268	38.3
9	106	21.2
10	61	22.6
		Mean 25.8

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

18 - 19 Weeks Post Infection Experiment

Daily Plasma Clearances

Rabbit No. 688 - Infected

Day	Faecal Activity	Clearance (ml)
1	940	8.6
2	1,302	16.5
3	1,333	21.2
4	1,134	22.7
5	784	19.1
6	584	17.7
7	270	10.4
8	374	17.0
9	230	13.5
10	263	18.8
		Mean 16.6

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

18 - 19 Weeks Post-Infection Experiment

Daily Plasma Clearances

Rabbit No. 63 - Infected

Day	Faecal Activity	Clearance (ml)
1	907	5.0
2	3,192	27.3
3	2,071	29.2
4	1,422	30.9
5	880	32.6
6	465	27.4
7	195	17.8
8	110	15.7
9	98	24.5
10	81	27.0
		Mean 23.7

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

18 - 19 Weeks Post-Infection Experiment.

Daily Plasma Clearances

Rabbit No. 56 - Control

1	54	0.61
2	238	3.5
3	155	3.0
4	166	4.3
5	103	3.6
6	105	4.8
7	123	7.2
8	87	6.7
9	32	3.2
		Mean 4.1

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

18 - 19 Weeks Post-infection Experiment

Daily Plasma Clearances

Rabbit No. 177 - Control

Day	Faecal Activity	Clearance (ml)
1	144	1.8
2	157	2.3
3	146	2.9
4	102	2.8
5	96	3.7
6	69	3.6
7	43	2.9
8	30	4.5
9	26	3.3
		Mean 3.1

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

18 - 19 Weeks Post-Infection Experiment

Daily Plasma Clearances

Rabbit No. 197 - Control

Day	Faecal Activity	Clearance (ml)
1	202	2.4
2	232	3.5
3	158	3.2
4	128	3.5
5	86	3.0
6	55	2.5
7	45	2.6
8	61	4.7
9	37	3.7
10	28	3.5
		Mean 3.3

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

18 - 19 Weeks Post-Infection Experiment

Daily Plasma Clearances

Rabbit No. 199 - Control

Day	Faecal Activity	Clearance (ml)
1	173	2.6
2	125	2.7
3	108	3.4
4	74	3.4
5	50	3.3
6	37	3.7
7	23	3.3
8	22	4.4
9	20	6.7
		Mean 3.7

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

18 - 19 Weeks Post Infection Experiment

Daily Whole Blood and Red Cell Clearances

Rabbit No. 095 - Infected

Day	Faecal Activity	Whole Blood Clearance (ml)	Red Cell Clearance (ml)
1	11,326	7.01	1.86
2	20,031	14,92	4.33
3	15,128	13.60	3.67
4	16,297	17.26	4.79
5	11,742	15.13	4.16
6	10,550	16.23	4.70
7	6,072	11.14	3.43
8	8,750	18.94	6.05
9	4,321	11.43	3.51
10	2,842	8.74	2.81
		Mean 13.4	Mean 3.9

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

18 - 19 Weeks Post-Infection Experiment

Daily Whole Blood and Red Cell Clearances

Rabbit No. 088 - Infected

Day	Faecal Activity	Whole Blood Clearance (ml)	Red Cell Clearance (ml)
1	8,256	5.16	1.25
2	15,901	12.05	2.95
3	17,027	15.76	3.85
4	14,321	15.91	3.96
5	9,912	13.39	3.43
6	7,986	12.88	3.37
7	4,930	9.86	2.67
8	5,500	13.09	3.57
9	2,880	8.47	2.31
10	3,662	12.63	3.51
		Mean 11.9	Mean 3.1

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

18 - 19 Weeks Post-Infection Experiment

Daily Whole Blood and Red Cell Clearances

Rabbit No. 63 - Infected

Day	Faecal Activity	Whole Blood Clearance (ml)	Red Cell Clearance (ml)
1	6,065	2.69	0.85
2	4,066	1.98	0.63
3	20,522	11.08	3.50
4	14,627	8.76	2.77
5	11,062	7.37	2.28
6	7,254	5.47	1.69
7	5,098	4.24	1.35
8	4,733	4.35	1.38
9	5,096	5.22	1.62
10	5,165	5.89	1.81
		Mean 5.71	Mean 1.79

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

18 - 19 Weeks Post-Infection Experiment

Daily Whole Blood and Red Cell Clearances

Rabbit No. 56 - Control

Day	Faecal Activity	Whole Blood Clearance (ml)	Red Cell Clearance (ml)
1	70	0.03	0.01
2	399	0.19	0.06
3	230	0.11	0.04
4	77	0.04	0.01
5	214	0.12	0.04
6	282	0.15	0.05
7	312	0.20	0.06
8	384	0.27	0.09
9	112	0.08	0.03
		Mean 0.13	Mean 0.04

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

18 - 19 Weeks Post Infection Experiment

Daily Whole Blood and Red Cell Clearance

Rabbit No. 177 - Control

Day	Faecal Activity	Whole Blood Clearance (ml)	Red Cell Clearance (ml)
1	-	-	-
2	245	0.09	0.04
3	144	0.06	0.02
4	260	0.11	0.04
5	192	0.09	0.03
6	138	0.07	0.03
7	144	0.07	0.03
8	126	0.06	0.03
9	156	0.08	0.03
		Mean 0.08	Mean 0.03

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

18 - 19 Weeks Post-Infection Experiment

Daily Whole Blood and Red Cell Clearance

Rabbit No. 197 - Control

Day	Faecal Activity	Whole Blood Clearance (ml)	Red Cell Clearance (ml)
1	356	0.13	0.05
2	522	0.19	0.08
3	432	0.17	0.07
4	352	0.14	0.06
5	202	0.09	0.03
6	239	0.11	0.04
7	230	0.11	0.04
8	306	0.15	0.05
9	179	0.09	0.03
10	133	0.07	0.02
		Mean 0.13	Mean 0.047

^{95}Nb Labelled Albumin / ^{51}Cr Labelled Erythrocytes

18 - 19 Weeks Post-Infection Experiment

Daily Whole Blood and Red Cell Clearance

Rabbit No. 199 - Control

Day	Faecal Activity	Whole Blood Clearance (ml)	Red Cell Clearance (ml)
1	125	0.05	0.02
2	144	0.06	0.02
3	298	0.13	0.05
4	68	0.03	0.01
5	11	0.006	0.002
6	106	0.07	0.02
7	120	0.07	0.02
8	140	0.08	0.03
9	110	0.07	0.02
		Mean 0.06	Mean 0.02